Alcohol, Alcoholism, and the Neural Correlates of Emotion

by

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Providence, Rhode Island

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Junior Investigator Award at the International Conference on Applications of Neuroimaging to Alcoholism (2008)

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RESEARCH EXPERIENCE

- **National Institutes of Health, National Institute of Alcohol Abuse and Alcoholism, Laboratory of Clinical and Translational Studies, Section of Brain Electrophysiology and Imaging** *(2005- present)*
  - Currently working with Dr. Daniel Hommer, using functional magnetic resonance imaging and other physiological and psychological measures to examine several aspects of drug and alcohol dependence, including:
    - the effect of acute alcohol administration on neural circuits underlying reward, motivation, and the processing of emotional images
    - the relationship of stress and anxiety to alcohol dependence

- the effects of drugs and alcohol on physical brain structures, gray and white matter growth and shrinkage, and intracranial volume
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- Duties include: designing and implementing protocols, designing and programming experiments, patient recruitment, running MRI scans on inpatients and healthy controls, analyzing data using Analysis of Functional Neuroimaging (AFNI), writing papers, extensive collaboration with doctors, nurses, technicians, social workers, staff scientists, and research assistants.

- **Brown University Department of Neuroscience** *2004- 2005*
  - Rotated in the laboratory of Dr. Sheila Blumstein, examining linguistic processing in patients with Broca's aphasia.
  - Designed and performed study to examine the role of semantic and lexical competition, and their interaction, on speech perception in a lexical-decision task.

- **Tufts University Department of Psychology**
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    - Designed and completed study comparing brain activity of participants when completing a task in a native vs. a novel language in order to examine the role of visual expertise.
  - Independent Study Project: Effect of Familiarity and Categorical Processing on Recall and Recognition Performance and False Memory *Fall 2003*
    - Designed and completed a study comparing performance on recall and recognition memory tasks when subjects were given highly familiar, categorical vs. novel, non-categorical stimuli.
  - Research Assistant: Investigating the Correlation Between Alcohol and Aggression in Squirrel Monkeys *Spring 2003*
    - Assisted in behavioral coding of squirrel monkey colonies, and helped analyze data examining how drugs affect dominance hierarchies.
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- **New York University Center for Neural Science** *Summer 2003*
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- **New England Medical Center** *3/02-8/02*
  - Worked in Autism Genetics Research Group to identify autism related genes
  - Collaborated with research team, compiled data, transcribed and analyzed interviews with both autistic and speech-impaired patients.
- **UCT International Laboratories**: Melville, NY *Summer 2001*
  - Tested effects of newly developed antibiotics on bacteria, performed quality control experiments, plated bacteria, and measured results.
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SELECTED RESEARCH PAPERS / PRESENTATIONS

Peer-reviewed Journal Articles

Gilman, J., Ramchandani, V., Bjork, J., Davis, M., and Hommer, D. Why we like to drink: An fMRI study of the rewarding and anxiolytic effects of alcohol. *(Journal of Neuroscience, in press).*

George, D.*, **Gilman, J**.*, Hersh, J.*, Thorsell, A.*, Herion, D., Geyer, C., Peng X., Rawlings R., Gehlert, D., Tauscher J., Hommer, D., and Heilig, M. (*these authors contributed equally). Neurokinin 1 receptor antagonism as a possible therapy for alcoholism. *Science,* Mar 14; 319(5869):1536-9.

Gilman J. and Hommer D. Modulation of brain response to emotional images by alcohol cues. *(Addiction Biology, in press).*

Gilman, J., Bjork, J., Hommer D. Parental alcohol use and brain volumes in early and late-onset alcoholics. *Biological Psychiatry*, 2007, Sep 15; 62 (6):607-15.

** This study was featured in United Press International, Medical News Today (UK), Science Daily, DG News, eMaxHealth.com, Spero News, WebWire, EurekAlert, and at least 30 other news sources.*

Hommer D., Momenan R, **Gilman J.** Gender and brain shrinkage in alcoholism. *Alcoholism- Clinical and Experimental Research* 31 (6): 107A-107A Suppl. S JUN 2007.

Gilman, J., Davis, M., and Hommer, D. Cognitive and emotional processing of affective stimuli in alcohol-dependent patients and healthy controls. *(in prep).*

Conference Poster Presentations

Gilman, J. and Hommer, D. How do alcohol cues influence emotional processing in alcohol-dependent patients? *Human Brain Mapping*, Chicago IL 2007.

Gilman, J. Salloum, J., and Hommer, D. Emotional processing of high-arousal positive and negative images in alcohol-dependent patients. *Society for Neuroscience*, Atlanta, GA 2006.

Gilman, J., Bjork, J., and Hommer, D. Influence of parental alcohol use on brain volumes in alcoholics. *Human Brain Mapping*, Florence, Italy 2006.

Steingrinsson, R., **Gilman, J**., Ramos, E., Majaj, N., & Pelli, D. Where are letters learned? An fMRI study. NYU Natural Sciences Poster Session, 2003. New York University, New York, NY, June 2003. (Can be viewed at www.psych.nyu.edu/pelli/steingrimsson2003nyu512.gif.)

Invited Lectures

Pharmacological fMRI and Alcohol Dependence: Applications, Experiments, and Limitations.
Delivered Feb 6, 2008, at headquarters of *Eli Lily and Company* in Indianapolis, IN.

Effect of Acute Alcohol Administration on Mesocorticolimbic Brain Structures in Social Drinkers.
Delivered Tues, Nov 6, 2007 at *Society for Neuroscience* conference in San Diego, CA.

Modulation of Brain Response to Emotional Images by Alcohol Cues.
Delivered on July 8, 2007, at the *Research Society of Alcoholism* conference in Chicago, IL.

How Does Parental Drinking Influence Intracranial Volume in Adult Alcoholics?
Delivered on Sept 8, 2007, at the *NIH Graduate Student Retreat.*
**This abstract was also selected as a finalist at the NIH Graduate Student Research Symposium in May, 2006, and was featured in the May-June 2006 issue of the NIH Catalyst.*

Defining a Letter Area: Using fMRI to Examine the Effect of Learning on Neural Specialization of Visual Letter Recognition.
Delivered Apr 23, 2004 at the *Tufts Undergraduate Research Symposium* in Medford, MA.

INTERESTS AND ACTIVITIES

Foundation for Advanced Education in the Sciences *2006- 2007*
Team-taught course on experimental techniques. Delivered lectures on brain imaging techniques including MRI, fMRI, PET, MEG, EEG, and ERP.

Graduate Student Council Member *2006- present*

Committees Include:

- *Graduate Student Symposium Committee*: organized symposium in which NIH graduate students presented posters, delivered talks, invited outside speakers, etc.
- *Graduate Student Retreat planner:* organized 2-day retreat for graduate students to present work, learn about the graduate partnership program, discuss career options, challenges in science, and various aspects of the graduate programs.
- *Interview Committee:* contacted prospective NIH graduate students from Brown, answered questions, organized meals, and guided them through the Brown/NIH interview process.

- *Social Committee:* plan and organize various social activities among graduate students including the annual Graduate Student Holiday Party.

Writing Fellow, Brown University Writing Center *2005-2006*

Tutored undergraduate and graduate students in writing papers, emphasizing structure, organization, grammar, content, and style. Ran writing skills workshops.

Tufts University Admissions Alumni Program Interviewer *2005*

Schedule meetings with prospective Tufts students. Conduct interviews, write evaluations of students to be used in the admissions process.

The Observer, Tufts' Student Magazine *2001-2004*

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Managed staff, ran meetings, generated ideas, wrote, assigned and edited articles, communicated with writers, lead workshops on writing skills and visual layout strategies, produced issues using Adobe Photoshop and Adobe InDesign.

Preface

The relationship between alcohol and emotion is extremely complex. Prolonged heavy alcohol consumption and acute alcohol administration both have profound impacts on emotional processing in the brain. In this thesis, we use functional magnetic resonance imaging to explore the interaction among alcohol, alcoholism, and the neural correlates of emotion. Chapter 1 provides background information on alcoholism and reviews the relevant literature that has guided our experiments. Specifically, I discuss why we may see differences in the emotional circuits of alcoholic patients relative to healthy controls, and suggest explanations for why alcoholic patients may use alcohol to neutralize these differences. Chapters 2, 3, and 4 describe original research that addresses differences in emotional response between alcoholic patients and controls, and attempt to modulate those differences through conditioned cues, pharmacological treatment, and task requirements. In Chapter 5, we administer alcohol intravenously to social drinkers, and demonstrate enhancement of positive affect and reduction of negative affect in the brain. Chapter 6 examines a possible genetic and/or environmental risk factor for alcoholism, reduced maximal brain growth, in order to determine if some deficits faced by alcoholic patients are pre-determined before the onset of heavy drinking. Finally, Chapter 7 provides a summary of results, implications of these results, and possible future directions.

Acknowledgements

My research would not have been nearly as successful, or as much fun, without the support and guidance of my advisors, family, and friends. First, I would like to thank my thesis advisor, Daniel Hommer, for all of his support, guidance, and encouragement throughout my time at NIAAA. I would also like to thank Vijay Ramchandani and Mike Kerich, because without them, nothing would ever work. I would also like to thank the many research assistants, nurses, and coordinators for all of their help in making this research possible. I would also like to thank the members of my thesis committee, Sheila Blumstein, David Sheinberg, and Lance Bauer, for agreeing to take the time to read and comment on this thesis.

I would like to thank my family, especially my parents, for their love and encouragement. Finally, I would like to thank all of my friends for their amazing support. Tara, Talia, Rachel, Emily, Christie, Lauren, Erica, and especially Tony, thank you for everything.

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Chapter 1:

Introduction

From a neuroscience perspective, alcoholism is a fascinating disease to study. Like other psychiatric diseases, it can cause serious health-related, job-related, and personal problems, and yet it is the individual's own behavior that propagates the disease. The compulsion to continue alcohol use despite increasingly negative consequences suggests that the brains of alcoholic patients may be different from those of people who are not addicted. The neural circuitry involved in the processing of emotion, reward, and motivation may underlie these differences. In this thesis, a series of experiments will be conducted to investigate the relationship among alcohol, alcoholism, and the neural correlates of emotion.

1. Introduction

According to the National Epidemiologic Survey on Alcohol and Related Conditions, almost 9% of the adult population of the United States (17.6 million) has met the criteria for an alcohol use disorder (AUD) in the past 12 months, while the lifetime prevalence of any AUD is 30.3% (Grant et al 2004) . This rate persists despite severe consequences associated with excessive alcohol use, such as increased motor vehicle crashes (Anda et al 1988), domestic violence (Leonard and Eiden 2007), risky sexual behavior (Corte and Sommers 2005; Deardorff et al 2005; Morojele et al 2006), economic costs and lost productivity (Pallarito 1995), as well as alcohol-related injuries (Cherpitel 2007) and other detrimental effects on health and disease (Dyson 2006; Epstein et al 2007; Lown et al 2007; McIntyre et al 2007; Rosenbloom et al 2007). And yet, despite the consequences of continued alcohol use, alcoholic patients report that they drink because they enjoy the sensation of euphoria and the reduction of stress and negative emotion associated with alcohol consumption (Woody 1992). Alcohol's ability to modulate mood states, combined with its wide availability and legality, renders it one of the most highly abused substances on the planet.

According to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), AUDs are split into categories of alcohol abuse and alcohol dependence (American Psychiatric Association (2000)). Diagnostic criteria for these disorders are shown in **table 1.1.** People who fulfill criteria for AUDs often have deficits in the natural regulation of mood and emotion. High comorbidity exists between AUDs and mood, anxiety, and personality disorders. Alcoholic patients are 3.4 times more likely than those without AUDs to have a mood disorder, 3.0 times more likely to have an anxiety disorder, and 3.4 times more likely to have a personality disorder (including 5.4 times more likely to have antisocial personality disorder). Whereas the twelve-month prevalence of mood and anxiety disorders among people without a substance use disorder is 9.21% and 11.08% respectively, the prevalence for patients with a substance use

disorder (including but not exclusively alcohol) is 19.67 % and 17.71% (Grant et al 2004).[1] Treatment-seeking alcoholic patients have a much higher prevalence; 40.7% are diagnosed with at least one mood disorder, and 33% with an anxiety disorder (Grant et al 2004). In the National Institute on Alcohol Abuse and Alcoholism (NIAAA) inpatient unit at the Clinical Center, 80.87% of patients are diagnosed with a mood and 40.5 % with an anxiety disorder (**Fig. 1.1**).

The objective of this thesis is to investigate the complex relationship among alcohol, alcoholism, and emotional dysregulation. In a series of experiments, functional magnetic resonance imaging (fMRI) is used to explore differences between alcoholics and healthy controls in the brain circuits that activate in response to both negatively valenced and positively valenced visual stimuli. The purpose of these experiments is to characterize the neural response to negative and positive images, and attempt to modulate this response through experimental and pharmacological manipulations. Next, the effect of alcohol itself on the neural circuits involved in the processing of emotional stimuli is investigated, in order to better understand how alcohol can alter emotional processing, and how this can lead to increased alcohol use and abuse. In the final experiment, we explore a possible genetic-environmental risk factor of alcoholism, reduced brain size, by using structural MRI to examine brain size and brain growth in alcoholics with and without a family history of heavy alcohol use.

[1] The *DSM-IV* allows for the diagnosis of both independent and substance-induced mood and anxiety disorders. The diagnosis of an independent disorder requires that (1) the mood or anxiety syndrome is established before the onset of substance use, and (2) that the syndrome persists for more than 4 weeks after the cessation of intoxication or withdrawal. Substance-induced disorders, on the other hand, occur only during periods of active substance use.

1.1. Why might alcoholics experience more negative emotion?

Several lines of evidence suggest differences between alcoholics and healthy controls in their neural response to emotional stimuli. The comorbidity of alcoholism and emotional dysregulation indicates that the neural circuits involved in emotional processing may be compromised in alcoholics. According to Koob et al (2008), addiction has three characteristics: compulsion to seek and take the drug, loss of control in limiting intake, and the emergence of a negative emotional state, such as dysphoria, anxiety, and irritability when access to the drug is prevented. Koob states that the negative emotional state is caused by two factors: (1) underactivation of natural motivational brain circuits such that the reward system becomes deficient, and (2) recruitment of the "antireward" system. Patients suffering from alcoholism have intense craving for the drug, and this craving can be driven either by negative or positive emotional states. Alcoholics often experience severe emotional and somatic withdrawal if the craving is not satisfied (Koob and Le Moal 2008).

Alcohol, like most other drugs of abuse, activates the mesocorticolimbic reward circuit, which includes the orbitofrontal cortex, amygdala, and ventral striatum/nucleus accumbens (NAcc), as well as the prefrontal cortex and the anterior cingulate (Knutson et al 2003; Sanfey et al 2003; Ullsperger and von Cramon 2003). A variety of primary and secondary rewards have been shown to activate this circuit, such as fruit juice and water (Berns 2001; O'Doherty 2002; Pagnoni 2002; McClure 2003), pleasant smells (Gottfriend 2002; Anderson 2003), and sexual stimuli (Arrow 2003), as well as conditioned rewards such as positive feedback and money (see Knutson 2005 for review). Studies have shown that drugs of abuse can acutely sensitize the activity of the mesocorticolimbic circuit. Most of this evidence comes from rat studies in which researchers implant electrodes in the NAcc, and then train the animals to press a lever to self-administer a current into this region that induces a pleasant or reinforcing feeling. Researchers can then establish a reward threshold for the rats, which is equal to the amount of current the rat needs in order to continue pressing the level to self-administer the current. Below that threshold,

the animal no longer finds the stimulation rewarding. Drugs such as cocaine (Vorel et al 2002) and alcohol (Lewis and June 1990) reliably decrease reward thresholds in healthy animals, perhaps by increasing the amount of pleasure obtained from a given amount of current.

During withdrawal, there is a decrease in the activity of the mesocorticolimbic system, which correlates with a decrease in opioid peptides, GABA, and glutamate in the nucleus accumbens and the amygdala (Koob 1992), and these reduced neurotransmitter levels can persist long after the abstinence. This modulation of reward circuitry following drug dependence has also been shown in humans. Volkow et al demonstrated a reduction of dopamine D2 receptors in alcoholic patients relative to controls, suggesting hypodopaminergic functioning and hypoactivation of the orbitofrontal and limbic cortex during abstinence (Volkow et al 2003; Volkow et al 1988). This decreased D2 receptor availability persisted even when patients were abstinent from alcohol for 1-4 months (Volkow et al 2002). Volkow suggests that these decreases in reward neurotransmitter function lead to a decreased sensitivity to the reinforcing properties of natural, non-drug-related stimuli in addicted patients, rendering them more likely to continue to use the drug. She proposes that in the addicted patient, the value of the drug and its associated cues is enhanced while that of other reinforcers is decreased (in part due to the higher intrinsic reward properties of drugs of abuse: increases in DA induced by drugs in the NAcc are three- to fivefold higher than those of natural reinforcers (Wise 2002)). The increased reward value of drugs causes a "resetting" of reward thresholds, so that acquisition of the drug becomes the main motivational drive for the individual. This decreased sensitivity to natural rewards may contribute to the negative emotional state associated with abstinence, and may also make recovering alcoholics more vulnerable to relapse (Koob and Le Moal 2008).

In addition to dysfunction of the reward system, Koob argues, the antireward system is recruited. The antireward system is a concept based on the hypothesis that there are systems in the brain which function to limit reward, and as dependence and withdrawal develop, these systems such as corticotrophin-releasing factor (CRF), norepinephrine, and dynorphin activate.

The hypothalamic-pituitary-adrenal (HPA) axis and the brain stress systems, which are both mediated by CRF, are dysregulated in addicted patients, which leads to an increase in adrenocorticotropic hormone (ACTH), corticosterine, and CRF during withdrawal (Koob 2008). During alcohol withdrawal, CRF systems become hyperactive, and there is evidence of increased CRF in the amygdala of alcohol-dependent rats (Funk et al 2006; Merlo Pich et al 1995). This may contribute to the enhanced anxiety associated with withdrawal.

The recruitment of the antireward systems cause a stress-like, aversive state, while at the same time, the underactivity of the motivational circuits (involving the ventral striatum and extended amygdala) decreases positive affect. Together, these phenomena potentiate a negative emotional state that, in turn, may intensify the desire for alcohol. The relationship between negative emotion and alcoholism, however, is complex. Although there is evidence that the alcoholism causes negative emotional states, it is also likely that individuals with pre-existing emotional disorders are more likely to self-medicate with alcohol and other drugs. In fact, drug-addicted patients typically experiment with several drugs before choosing a particular drug to manage specific painful affective states or psychiatric disorders (i.e. patients with depression, alexithymia, and hypomania often choose stimulants for their energizing properties; patients with excessive rage or aggression choose opiates; patients who are disengaged from feelings and relationships choose alcohol because it permits the experience of affection, closeness, or aggression) (Khantzian 1997). The prolonged negative affect experienced by alcoholics is most likely caused by a combination of pre-existing characteristics that cause the individual to initiate drug use, and drug-induced changes that cause the individual to continue using. Personality, environmental factors, and genetic and epigenetic mechanisms also contribute to alcoholism and other drug addictions.

The purpose of this report is not to distinguish between the causes of emotional dysregulation in alcoholics, but rather to understand the brain circuitry underlying them, and to explore ways to modulate these circuits.

1.2. Measuring emotion

Emotion is notoriously difficult to study, mainly because the precise definition of emotion is controversial. Many researchers agree that emotion is composed of two elements; valence, or how negative or positive one feels, and arousal, or how intensely one experiences the negative or positive emotion.

Arousal is significantly easier to characterize than valence. The skin conductance response is known to reflect sympathetic activity, and can be used to measure excitement, emotional reactivity, or tension. Many studies use measures of heart rate, blood pressure, pupillary dilation, and levels of cortisol or adrenocorticotropic hormone (ACTH) to assess arousal.

The valence dimension of emotion is more complicated to physiologically characterize. Valence is driven by one of two opposing motivational systems; "appetitive" motivations linked to approach behaviors, and "aversive" motivations linked to avoidant behaviors. Some theorists claim that optimal arousal engenders appetitive states, whereas hyperarousal induces aversive states, implicating one arousal continuum that can account for both appetitive and aversive motivations (e.g., (Gray 1987). Others, however, have argued that separable neural substrates underlie the perception of positive and negative emotions. For instance, LeDoux has proposed that a system mediating aversive motivation runs from the amygdala through the stria terminalis to the medial hypothalamus and periaqueductal gray (LeDoux 1996), while Depue and his colleagues have proposed that dopaminergic ventral tegmental projections to the nucleus accumbens and medial prefrontal cortex mediate the experience of appetitive motivational states (Depue et al 1994). Although precise valence measurements are difficult to make because of individual variance, emotion has been systematically measured using EMG activity (i.e. facial-muscular patterns), startle-reflex patterns, and hemispheric brain activity. In this section, I will focus on the brain activity methodology of differentiating emotional valence.

Functional MRI is emerging as an important technique in the study of emotional processing and the dysregulation of emotional circuitry.[2] Results of previous studies have indicated that the activity of subcortical areas implicated in motivational processes can be measured in this way. Presentation of pleasant or unpleasant stimuli compared to neutral stimuli have been shown to reliably activate brain regions involved in emotional processing, such as the amygdala (Breiter et al 1996; Liberzon et al 2003; Morris et al 1996), hippocampus and parahippocampal gyrus (Gur et al 2002; Lane et al 1997c), insula (Phan et al 2003; Phillips et al 1997), anterior cingulate (Herwig et al 2007; Mohanty et al 2007; Nieuwenhuis et al 2005; Rota et al 2007), medial and ventromedial prefrontal cortex (Bufkin and Luttrell 2005; Erk et al 2006; Harenski and Hamann 2006; Kim et al 2003; Lane et al 1997b), and visual cortex (Liberzon et al 2003; Morris et al 1998; Vuilleumier 2005). Studies have found that the amygdala preferentially responds to disturbing versus neutral and pleasant pictures (Irwin et al 1996), and that it preferentially responds to fearful versus neutral faces (Breiter et al 1996; Morris et al 1996). While most of these regions are involved in a variety of cognitive operations, these regions

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[2] fMRI is a systems-level approach to neuroscience that exploits the magnetic properties of brain tissue to generate images of brain function. This technique is advantageous because it provides excellent spatial resolution (< 1 millimeter), and very good temporal resolution (6-12 seconds), and is one of the only modern neuroscience techniques that is completely non-invasive and still allows the measurement of neural activity of the whole human brain. fMRI is based on the theory that there is a tight coupling between neuronal activity and regional cerebral blood flow. The most commonly used signal for fMRI is called the blood oxygen level-dependent, or BOLD, signal, which is driven by differences in magnetic properties of oxygenated and deoxygenated hemoglobin.

The fMRI scanner emits a constant magnetic field, called B_0, which is measured in units of tesla (T). Field strengths of research scanners commonly range from 1.5T to 7T. In the presence of B_0, hydrogen atoms in the brain align either parallel or antiparallel to the main magnetic field. After atoms are aligned, the scanner emits a radio frequency pulse into the neural tissue, which disrupts the alignment of the molecules, exciting them to a higher energy state (which is called resonance). Then, the coils in the scanner measure the time it takes for these molecules to return to their original resting state. The two time constants used in fMRI are T1 and T2. T1 measures relaxation in the direction of the magnetic field of B_0, while T2 measures relaxation perpendicular to B_0. Since protons lined up against the magnetic field require more energy than those lined up with the magnetic field, there will be an overall magnetic field in the direction of B_0. T2 is a time constant of the decay in an ideal, homogeneous magnetic field, but in physiological tissue, this constant is called T2*, because this decay is influenced by the local blood supply in the region.

T2* is the actual measure of the ratio of deoxyhemoglobin (dHb) to oxygenated hemoglobin. Neural activity in a region changes the blood flow and the oxygen usage in that region. This in turn changes the local magnetic field in that region. An "activated" brain area is one to which there is an increase in T2* relative to a baseline state or to a specific control brain state (Logothetis et al 2004). Activations in an fMRI study are generally reported as a percent signal change from that baseline condition in specific voxels (small 3-dimensional units of brain mass). fMRI only allows for the measure of relative signals, so an fMRI experiment must alternate between two or more conditions in order to look for the change in signal between the conditions.

appear to be more highly activated in response to emotionally negative or positive than to neutrally-valenced stimuli.

In addition, some researchers (e.g. Davidson, 1992) have proposed a regional asymmetry in brain activation, demonstrating that neural systems mediating approach behavior recruit the left frontal lobes, whereas withdrawal-related systems are governed by the right frontal lobes. Patients with damage to the left frontal lobe are apathetic, experience loss of interest and pleasure in objects and people, and have difficulty initiating voluntary action, or psychomotor retardation (Davidson 1992). Davidson suggests that hypoactivation in this region should be associated with experience more sadness and depression. Furthermore, patients with damage to the left hemisphere report greater dispositional negative affect (Canli et al 1998). Morris and colleagues have also shown that during the experimental arousal of withdrawal-related emotional states (e.g., fear and disgust), the right frontal and anterior temporal regions are selectively activated (Morris et al 1996). Davidson further suggests that these observations suggest a specialization for certain right hemisphere regions in the mediation of avoidance-related negative affect. Consistent with this theory, researchers found that the anxiolytic diazepam shifts frontal asymmetry toward a pattern of greater left-hemispheric activity in both monkeys (Davidson 1992) and in humans (Mathew et al 1985).

In the reported experiments, BOLD activity is used to measure activation in regions of the brain implicated in the processing of emotion (i.e. amygdala, parahippocampal gyrus, insula, anterior cingulate, prefrontal cortex, primary and secondary visual processing areas). Comparisons within and between groups should indicate whether there are identifiable and consistent differences between the response to positive and negative images within each group, and if there are observable differences between alcoholics and controls in the response to each stimulus class. After establishing differences, this technique provides a baseline allowing for the introduction of experimental manipulations, such as the presentation of drug cues, the

introduction of a pharmacological compound, or the requirement to perform a cognitive or emotional task, in order to investigate factors that may influence emotional brain circuitry.

1.3. Neuroimaging evidence of negative emotion in alcoholics

Despite research showing that alcoholics and other drug-addicted patients experience a persistent negative affective state, and despite the emergence of fMRI as a technique to study the neural circuits underlying emotional processing, very few studies have examined the neural circuits involved in emotional processing in alcoholism. Studies have shown that patients with other psychiatric disorders, such as depression, show increased brain activity in response to negative stimuli. Surguladze and colleagues reported increased activity in the right fusiform gyrus, ventral striatum, and left parahippocampal gyrus to sad faces in patients with major depressive disorder compared to healthy controls (Surguladze et al 2005). Depressed patients also show increased activation in cortical and limbic regions compared to healthy controls when viewing negative emotional slides (Anand et al 2005), and this increased activation can be attenuated after treatment with selective serotonin reuptake inhibitors (Fu et al 2004). (Although, it should be noted, another study of patients with major depressive disorder reported that patients relative to controls showed decreased activations in the dorsolateral prefrontal cortex, inferior and medial orbitofrontal cortex, caudate, and hippocampus, when shown pictures of sad faces (Lee et al 2008)). In alcoholism, Elkins and colleagues have demonstrated that alcoholics have a greater tendency to experience negative affect (Elkins et al 2006), and using a Stroop task, it has been shown that they have an attentional bias toward negative emotional words (Stormark et al 2000). The available neuroimaging data on alcoholics' responses to negative and positive stimuli, however, is somewhat inconsistent.

In a study by Heinz et al (2007), alcoholics demonstrated greater activation than controls to positive images in the ventral striatum, superior frontal gyrus, thalamus, and anterior cingulate, and it was reported that greater activation to positive images was associated with better clinical

outcomes for these patients. However, the alcoholics in this study also demonstrated greater activation than controls to negative images in the superior frontal gyrus and the medial frontal gyrus, suggesting a general hyperactivity of emotional brain circuits. In contrast, Salloum et al (2007) reported blunted anterior cingulate response to negative faces in alcoholics relative to controls in an emotional decoding task. Glahn et al (2007) found that even among non-alcoholic adults, participants with a family history of alcoholism had less amygdala activation to fearful faces than non-abusing family history negative participants. The conflicting findings suggest that the emotional response of alcoholics is not well understood. Further study is thus needed to improve our understanding of the brain response of alcoholic patients to emotional stimuli.

1.4. The effect of acute alcohol on the brain

To understand the role of emotional dysfunction in alcoholism, it is essential to understand how alcohol itself affects the brain circuitry involved in emotional processing. Do alcoholic patients choose to drink alcohol because it does indeed alter the neural correlates of emotional states? Alcohol may affect the brain circuits underlying emotional processing through its activity in the ventral striatum. While the pharmacology of alcohol is complex and incompletely understood, alcohol may increase the firing rate of dopaminergic neurons in the ventral striatum, causing a reduction in dysphoria and an enhancement of positive emotion. In the reported experiments, neutral and fear-inducing stimuli are presented to participants under intoxication and placebo conditions, in order to investigate how alcohol changes neural activation to different classes of stimuli.

To date, there have been no fMRI studies that have reported BOLD activity during acute ethanol exposure, but there are several studies that indicate that alcohol may activate the reward circuits in the brain. Positron emission tomography (PET) studies that have examined the effects of acute ethanol administration on regional glucose metabolism or blood flow in the human brain have demonstrated activations in the right prefrontal cortex (Tiihonen et al 1994), the left

temporal cortex and left striatal regions (Wang et al 2000), and the bilateral thalamus (Boecker et al 1996), and preferentially in the ventral stiatum (Boileau et al 2003). Shreckenberger and colleagues (2004) used intravenous (IV) alcohol infusion in a PET study using 18-FDG, and showed that IV alcohol stimulated the bilateral striatum and the frontal cortex, and deactivated the occipital cortex (Schreckenberger et al 2004). These studies indicate that local, regional effects of alcohol can be measured in the brain over a short time course.

While none of these studies used fMRI to measure the neural response to alcohol administration, previous studies have investigated how other drugs of abuse affect BOLD activity in regions of the brain. Stein et al (1998) found significant regional activation in the insula, frontal lobe (orbital, dorsolateral and medial frontal), occipital cortex, and amygdala as well and the hypothalamus and limbic thalamus, when nicotine was given to research participants during an fMRI scan. These areas are associated with cognition (attention and working memory) and emotion (motivation, mood and arousal). In an fMRI study with cocaine, Breiter et al (1997) demonstrated activation in the ventral tegmentum, pons, basal forebrain, caudate, cingulate and most regions of the prefrontal cortex. These activations correlated with subjective ratings of rush and high.

Although the ventral striatum has been accentuated as the primary area that will be found to be activated following alcohol administration, these studies indicate the ventral striatum is not necessarily the only area that may be activated following drug taking. We plan to investigate the response to alcohol not only in striatal regions, but also in emotional-visual regions such as the amygdala, parahippocampal gyrus, and lingual gyrus, in order to understand how alcohol might modulate negative as well as positive emotional states. No previous studies, to our knowledge, have used fMRI to examine the response to alcohol during the presentation of emotional stimuli. Since the striatum has connections with limbic areas implicated in emotional processing (Groenewegen et al 1999), it is possible that the striatal response to alcohol could be influenced by emotional cues.

1.5. Theories and experimental evidence on alcohol and emotion

Many researchers have taken a psychological rather than a biomedical approach to understanding the relationship between alcohol and emotion, and these theories are worth reviewing. Hull's model of self-awareness states that alcohol impairs the cognitive processing of self-relevant information, so therefore if information is unpleasant or uncomfortable, then alcohol will attenuate that discomfort (Hull 1981). According to this theory, individuals who are self-conscious will be more sensitive to self-relevant cues, and alcohol will attenuate the stress response of these individuals. This theory is based on Hull's findings, which suggest that highly self-conscious participants manifest greater autonomic responses (i.e. skin conductance response (SCR), finger pulse amplitude) than low self-conscious individuals during the anticipation of a stressful situation. Under the influence of alcohol, these differences are no longer significant. This suggests that alcohol may interfere with cognitive processing of stressful information, therefore relieving the participants of feelings of self-consciousness (Hull and Young 1983). This theory, however, has several limitations. The alcohol in this experiment could have directly modulated physiological responses in these subjects regardless of cognitive processing of information. It is possible that this effect is more apparent in the highly self-conscious individuals who have larger physiological responses. Furthermore, some other studies have failed to replicate these results (e.g. Sher et al 1986) and some have shown that low- self-conscious individuals were even more responsive to cues than highly self-conscious individuals (e.g. Niaura et al 1988). Finally, intersubject differences in ability to describe emotion could have confounded this study. Alexithymia, a condition is which a person has little ability to describe emotions, may have been more prevalent in one group. For instance, alcoholics on average tend to be alexithymic (Ziolkowski et al 1995).

A second model that has emerged is Steele and Joseph's attention-allocation model (1986), which states that alcohol decreases anxiety by impairing all cognitive activity that requires effortful processing. According to this model, alcohol prevents anxiety by restricting an

individual's attention to the most salient and immediate cues. A caveat of this model is that if alcohol alleviates stress by distracting the individual from deep cognitive processing, then the distracting activity must be either positively valenced or neutral (Steele and Josephs 1988). If the distraction is negative, then alcohol may instead increase negative affect. This theory, however, has not been empirically tested. To our knowledge, studies have not yet investigated valence and attentional demands under intoxication conditions.

Finally, a third model assessing the effect of alcohol on emotion is the appraisal-disruption model (Sayette 1993), which states that alcohol downregulates the appraisal of stressful information by interfering with the spread of activation of associated information in long-term memory. According to this theory, alcohol diminishes the ability of a stressor to activate memories and concepts in long-term memory that are associated with the stressor, so that the intoxicated person cannot make the associations he or she would make when sober. This model predicts that alcohol will only have a stress-dampening effect if the individual learns about a stressful event while he or she is already intoxicated, and not before drinking occurs. Sayette and colleagues designed an experiment that exposed participants to a stressor either before drinking or after they were intoxicated. In the condition in which participants learned of a stressor before drinking, investigators failed to find any stress-response dampening effects of alcohol (Sayette 1993), and previous studies have even reported an enhanced stress response (e.g. Keane et al 1980). In contrast, if participants found out about an upcoming stressor while they were intoxicated, then their physiological stress response was attenuated (Sayette 1993). Although this evidence supports the theory that alcohol may constrain the ability to associate stressful events with negative information stored in long term memory, we still do not know if it selectively inhibits the spread of negative information while failing to affect positive information.

Most psychological studies of alcohol and emotion have only examined the arousal response, ignoring the valence dimension of emotion. These experiments have typically involved exposing the participant to a physical or mental stressor under the influence of alcohol, and

measuring physiologic stress responses. The problem with these studies is that because there is only a negative high-arousal state, if alcohol is found to decrease the stress response, it is unclear as to whether alcohol is affecting the reaction to aversive stimuli or the reaction to all emotional conditions (Stritzke et al 1996).

Stritzke and colleagues designed an experiment in which participants received either alcohol or placebo before viewing negative, positive, and neutral pictures. In this study, alcohol diminished the magnitude of the startle response and the skin conductance response regardless of the valence of the stimuli, suggesting that alcohol diminished overall emotional reactivity. In another study by Gabel at al (1980), in which alcohol was the dependent variable, healthy participants viewed negative, positive, and neutral pictures and were then given the choice to drink alcohol. Both negative and positive stimuli resulted in increased arousal, but alcohol consumption was highest after the participants had viewed the positive slides. The amount of alcohol consumption following the negative slides was not statistically different from the amount consumed following neutral slides (Gabel et al 1980). Therefore, the anxiolyic properties of alcohol may not be selective to the repression of negative emotion. The increased alcohol consumed after exposure to positive images may indicate that the subjects in this study associated drinking with positive emotion. In addition, subjects may have believed that alcohol would enhance, rather than repress, emotion, and were therefore more willing to drink after viewing the positive pictures.

Curtin et al (1995) used the startle reflex as a measure of the effect of alcohol in a stressful situation. They presented sober and intoxicated individuals with cues that signaled either safety or a threat of electric shock. They found that autonomic activity, including SCR, heart rate, EMG, and magnitude of startle responses, were all decreased in the intoxicated condition, but they did not find an interaction between intoxication and threat condition. This study, like Gabel's and Stritzke's, suggests that alcohol diminishes overall autonomic nervous system activity, but does not selectively block either response to cues or selectively inhibit

stressful responses. Furthermore, since the intoxicated participants still show a fear-potentiated startle reflex, then it is unlikely that alcohol acts directly on the subcortical amygdala or the brainstem acoustic startle circuit, but instead acts nonspecifically on higher-order cognitive processing (Stritzke et al 1996).

In the current alcohol exposure experiments, if we see a reduction in the response to fear-inducing stimuli, it could provide evidence for either Steele and Joseph's attention-allocation model (i.e. participants are not deeply processing the fear-inducing stimuli), or for Sayette's appraisal-disruption model (i.e. the fearful stimuli fail to activate memories of negative experiences in long-term memory). Obtaining a measure of the physiological response to alcohol in the brain will further our understanding of alcohol's effect and emotion, and help to substantiate these theories.

1.6. Pharmacokinetics of Alcohol and Emotion

A major limitation of existing literature on alcohol's effects on emotion is that alcohol can have vastly different effects depending on at least three factors: the point on the blood alcohol curve in which an experiment is conducted, the individual's drinking history, and the dose of alcohol administered. Several researchers have postulated that alcohol acts as a stimulant on the ascending limb of the blood alcohol curve (BAC) and a depressant during the descending limb, but studies have been inconclusive. King et al (2002) studied the biphasic effects of alcohol in light drinkers and heavy drinkers, and demonstrated heightened sensitivity to stimulant effects during rising BACs, and lowered sensitivity to sedative effects during falling BACs, in the heavy drinkers. In this study, the light drinkers reported a more stable response during the two phases of the BAC, with no increase in stimulation and overall heightened sedation throughout both limbs of the BAC. Conrod et al (2001) found that increases and decreases in heart rate correlated with subjective measures of feeling "energized" or "sedated" respectively, and like King and colleagues, found that alcohol causes more stimulation and less sedation for heavier drinkers.

However, these results could be due in part to the fact that heavy drinkers had developed a higher tolerance for the sedative-like effects of alcohol. Finally, Giacola and Zeichner (1997) found that the biphasic effects extend to aggression; in a paradigm where subjects administered shocks to a fictitious opponent, the subjects who gave the shocks during the ascending limb of the BAC acted more aggressively than both sober controls and the subjects who gave shocks during the descending BAC.

Other studies have shown that the dose of alcohol is more significant than the timing of administration in influencing the subjective mood effects of alcohol. Turkkan et al (1988) administered three ethanol doses to alcoholics and controls, and measured self-report ratings and physiological measured before, during, and 90 minutes after ingestion. They found that heart rate and temperature remained elevated after the peak of the BAC, and failed to see any biphasic response. Control participants reported feelings of euphoria after higher doses of alcohol, whereas alcoholics showed little change in affect even following the high dose (likely due to increased tolerance). Levenson et al (1980) conducted a similar experiment, and found that subjects reported more pleasure after higher doses of alcohol, and manifested a faster heart rate and prolonged pulse transmission well after the peak BAC level. These studies suggest that the absolute amount of alcohol may be more important than the point on the BAC in determining alcohol's effect on emotions.

Finally, other studies have found that it is a combination of the dosage and the time point on the BAC that determines alcohol's subjective mood effects. Lukas et al (1986) found increases in EEG alpha activity during the rising BAC that paralleled subjective feelings of euphoria, but this was only apparent at high alcohol doses. These studies cast doubt on the theory that alcohol acts as a stimulant at low doses but a depressant at high doses. A large body of anecdotal evidence, however, suggests that emotion varies throughout the time course of an intoxication episode, and differs as a function of dosage.

Since few studies have systematically controlled for these factors, we cannot come to any conclusions about these effects. In the current studies, we closely monitor these variables in order to better elucidate their contributions to the change in emotional states during alcohol administration. Using an intravenous ethanol solution and physiologically-based pharmacokinetic model for alcohol, we can limit inter-subject variability in the alcohol response and precisely control the time course of exposure to alcohol in the brain to alcohol. Tightly controlling these factors allows us to more accurately examine how emotional cues interact with alcohol in both striatal regions and in emotional-visual brain circuits.

1.7. Objectives

It is the goal of the the experiments in this dissertation to examine emotional circuits affected by alcoholism, and how alcohol itself affects those circuits. We first attempt to characterize differences in emotional processing between alcoholics and controls using visually affective images as emotional probes. We then investigate whether these differences can be modulated through experimental tasks including cue-based, pharmacological, and cognitive manipulations. We then attempt to more thoroughly understand how alcohol, the etiological agent of alcoholism, can alter the brain's emotional circuits. A more complete understanding of the complex interaction among emotion, alcohol, and alcoholism will ultimately provide invaluable insight into the disease, leading to better prevention efforts and better treatment options for clinicians.

Table 1.1. Diagnostic criteria for alcohol use disorders according to the DSM IV.

Alcohol Dependence	Alcohol Abuse
Individual has to meet three or more of the following occurring at any time during a 12-month period:	*Individual has to meet one or more of the following occurring at any time during a 12-month period.*
Tolerance (a need for increased amounts of the substance to achieve intoxication, or the desired effect, or markedly diminished effect with continued use of the same amount).	Recurrent alcohol use resulting in a failure to fulfill major role obligations.
Withdrawal (symptoms include sweating, increased pulse rate, hand tremor, nausea or vomiting, hallucinations, psychomotor agitation, anxiety, and seizures within several hours to a few days after cessation of/reduction in alcohol use).	Continued alcohol use despite having persistent or recurrent social or interpersonal problems caused/exacerbated by use of the substance.
Alcohol is used in larger amounts and for longer period than intended.	Recurrent alcohol-related legal problems.
There is a persistent desire or unsuccessful efforts to reduce or stop use.	Recurrent alcohol use in hazardous situations.
A great deal of time is spent obtaining, using or recovering from use of alcohol.	
Important activities reduced or given up because of alcohol.	
Alcohol use is continued despite problems caused or exacerbated by alcohol.	

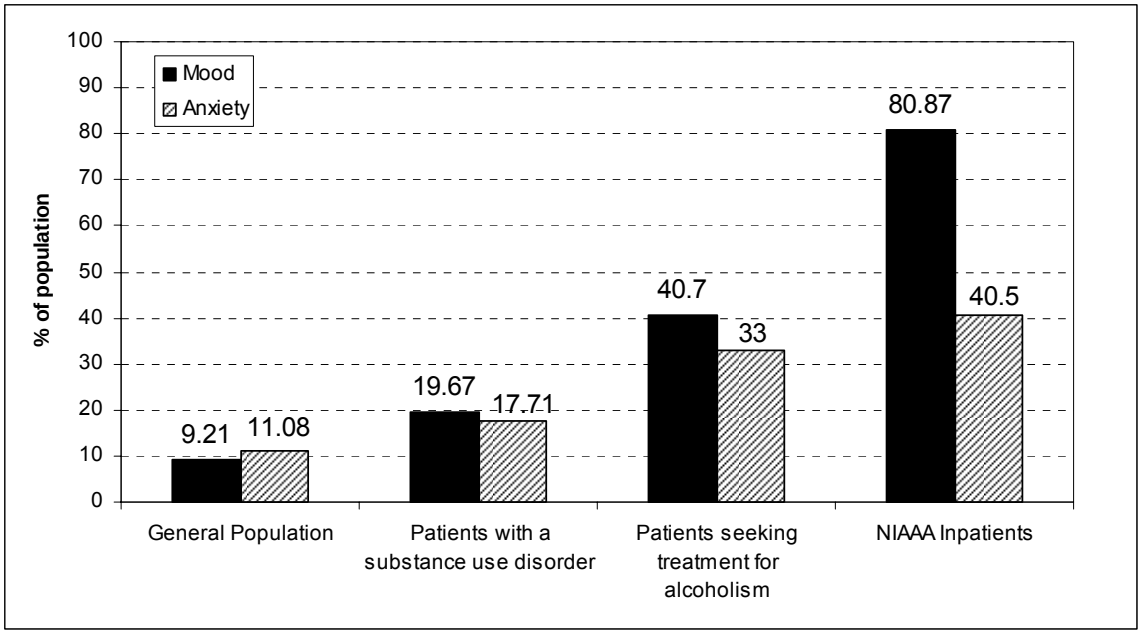


Fig 1.1. Twelve-month prevalence of mood and anxiety disorders. Data from the general population, patients with a substance abuse disorder, and patients seeking treatment for alcoholism was acquired from the National Epidemiologic survey on Alcohol and Related conditions (n = 43,093). NIAAA inpatient data was compiled from all of the patients admitted to the NIAAA inpatient unit from Jan 1996 to Sept 2004 (n = 413). Mood disorders include depression, dysthymia, mania, and hypomania. Anxiety disorders include panic disorder, social phobia, specific phobia, and generalized anxiety disorder.

Chapter 2:

Modulation of Brain Response to Emotional Images by Alcohol Cues in Alcohol-Dependent Patients

The purpose of this study is twofold. First, this experiment provides a preliminary understanding of differences between alcoholic patients and healthy controls in the processing of emotional visual images in specific brain regions. Second, this experiment will examine whether differences in processing of emotional visual images by the two groups of subjects can be modulated by conditioned stimuli. In this study, visual alcohol cues (or non-alcoholic beverage cues) are presented simultaneously with emotional images in order to understand how the alcohol cues interact with emotional cues. Our working hypothesis is that alcoholics will demonstrate greater activation than controls to the negative images in emotional-visual brain regions (such as the amygdala, parahippocampal gyrus, insula, fusiform gyrus, and lingual gyrus), and that the alcohol cues may decrease those differences.[3]

[3]Most of this chapter is taken from an *in press* manuscript: Modulation of brain response to emotional images by alcohol cues. **Gilman J**, and Hommer D. *(Addiction Biology, in press)*

2.1. Introduction

People often use drugs of abuse to regulate their moods, especially negative moods (Thorberg and Lyvers 2006). Alcohol drinkers report that they use alcohol both to enhance positive affect and to reduce dysphoria (Cooper et al 1995; Kassel et al 2000), and alcohol-dependent patients specifically state reduction of negative affect as a primary reason for drinking (Woody 1992). In addition, treatment-seeking alcoholic patients often suffer from comorbid psychiatric disorders, including major depression, dysthymia, phobias, generalized anxiety disorders, and panic disorders (Black et al 1987; Herz et al 1990; Hesselbrock et al 1985; Powell et al 1987; Ross et al 1988; Tomasson and Vaglum 1995). Thorberg and Lyvers found that mood self-regulation is impaired in substance abusers, (Thorberg and Lyvers 2006) and suggested that this impairment may predispose to substance abuse and addiction.

Compared to healthy individuals, patients suffering from depression, phobias, anxiety disorders, and panic disorders show an attentional bias toward negative or threatening stimuli compared to positive or neutral stimuli (Bradley et al 1997; Lang and Sarmiento 2004; Mathews et al 1996; Spector et al 2003). In a non-clinical population, participants with elevated trait anxiety scores also were less able to ignore negative stimuli (MacLeod and Rutherford 1992). A similar attentional bias has been noted among alcoholics. In a study by Stormark et al using the Stroop task, alcohol-dependent patients showed an attentional bias toward negative emotional words (Stormark et al 2000). This bias toward negative stimuli could be related to comorbid psychiatric disorders, alcoholism itself, or a combination of both.

In addition to biased attention to negative stimuli, patients with substance abuse problems have an attentional bias toward drug-related cues. This has been reported in individuals with alcohol (Bauer and Cox 1998; Cox et al 2002; Cox et al 1999; Ryan 2002; Sharma et al 2001; Stetter et al 1995; Stormark et al 2000; Townshend and Duka 2001), cocaine (Franken et al 2000a; Franken et al 2000b; Rosse et al 1997; Rosse et al 1993), opiate (Franken et al 2000b;

Lubman et al 2000), marijuana (Field 2005), and nicotine (Franken et al 2000b; Gross et al 1993; Johnsen et al 1997) dependence, as well as among caffeine users (Yeomans et al 2005). Robinson and Berridge suggest that stimuli associated with drugs of abuse become particularly salient, and therefore "grab attention" more than non-drug-related stimuli (Robinson and Berridge 1993). Drug cues have motivational significance since they predict a rewarding event to substance abusers, and therefore tend to dominate attentional resources.

This attentional bias to drug cues can been shown using brain imaging techniques. Several imaging studies have shown that specific brain regions are more highly activated by drug and alcohol-associated cues compared to neutral cues in drug users (Braus et al 2001; Breiter and Rosen 1999; Childress et al 1999; Garavan et al 2000; George et al 2001; Grant et al 1996; Maas et al 1998; Schneider et al 2001; Wexler et al 2001). Alcoholics show increased activation to visual alcohol cues in the anterior thalamus, prefrontal cortex (George et al 2001), anterior limbic regions, (Myrick et al 2004) and ventral putamen (Braus et al 2001), and a study using alcohol odors reported increased activity among alcoholics in the right amygdala, hippocampus, and cerebellum (Schneider et al 2001).

Functional imaging studies of alcoholics' response to negative, fear-inducing visual cues have not been conducted; however, there appears to be increased blood oxygenation level–dependent (BOLD) response to emotionally negative facial expressions among individuals with high trait anxiety (Stein et al 2007). Thus, it seems likely that alcoholics would show altered processing of fear inducing stimuli as well as stimuli associated with alcohol use. Preliminary studies in our lab suggested that alcoholics showed greater activation than controls to emotionally negative images, particularly in amygdala and temporal lobe. Based on our pilot data we designed a study to examine the interactions between emotionally arousing images (both positive and negative) presented simultaneously with alcohol or non-alcohol beverage images.

We used functional magnetic resonance imaging (fMRI) to examine brain activation in response to combination images which factorially juxtaposed negatively and positively valenced

emotional images with alcohol and non-alcohol beverages in both hospitalized alcoholics and healthy non-alcoholics. Several possibilities may occur when the alcohol cues are paired with the positive and negative images. A simple additive interaction could occur, where in alcoholics, the combination of both the alcohol cues and the negative images lead to a BOLD response greater than the response to either stimulus alone. However, a previous study that presented multiple stimuli in the visual field found that activation to each stimulus interacted in a suppressive fashion (Kastner et al 1998). Another possibility is a more complex interaction, where attention to one stimulus type affects the response to the other stimulus type (i.e. alcohol cues reduce activation to negative, but not positive, images). In control participants, we do not expect the beverage images to affect activation to the emotional images. In alcoholic patients, however, the alcohol beverage images may affect brain response to the negative or the positive images, both because the alcohol beverage cue is a strong distracter, and because the beverage cue may simulate the euphoric or anxiolytic response associated with the drug itself.

This study tests the following hypotheses: (1) alcoholics will show more activation in limbic regions and in the visual ventral processing stream to negative images than positive images (irrespective of with which beverage type they are paired); and (2) A higher-order emotion-valence by beverage type interaction will be evident where alcohol cues will modulate the response to negative images in alcoholics, but not in control participants. We also hypothesize that patients with higher anxiety scores will show increased activation to negative pictures, and this effect may also be modulated by the alcohol cues.

2.2. Methods

Participants

Twelve community-recruited healthy controls and 12 males with alcohol dependence participated. Alcohol-dependent patients were recruited from the National Institute of Alcohol Abuse and Alcoholism (NIAAA) inpatient unit at the Clinical Center of the National Institutes of Health in Bethesda, MD. We excluded patients with a history of delirium tremens or gross neurological disorders, an IQ less than 80, or who demonstrated signs of dementia or Korsakoff's disease. Patients were not thiamine deficient at admission, and did not have a history of head injury requiring hospitalization or any serious alcohol-related medical disorders. All participants were assessed with the Structured Clinical Interview for DSM-IV (First 1996), which confirmed that each patient met criteria for alcohol dependence. Patients were scanned three weeks after admission.

Healthy community-recruited age-matched male participants with no history of significant medical illness or psychiatric disorders were included for comparison. All participants were right-handed and had normal or corrected-to-normal vision. All participants provided written informed consent to participate in the study, which was approved by the NIH Neuroscience Institutional Review Board.

Participant demographic characteristics are provided in **table 2.1**.

Visual stimulation and task

Visual images were chosen from the International Affective Picture System (IAPS) (Lang 1995). Fifty-five high-arousal negative pictures and 55 high-arousal positive pictures were presented. These pictures were paired with pictures of alcoholic beverages and non-alcohol beverage pictures (i.e. milk, orange juice). The IAPS pictures and the beverage pictures appeared simultaneously, side-by-side (see **fig. 2.1** for examples). Scrambled images were used as the control condition and were displayed during the inter-stimulus-interval (ISI). The scrambled images were derived from the IAPS images using a script that introduced a random phase shift

into Fast Fourier Transformations (FFT) of each image, which preserved the overall brightness and color as the original image but no did not contain recognizable features. The presentation of the stimuli was jittered so that the inter-stimulus interval (ISI) ranged from 0 sec (i.e. one picture was presented immediately after another) to 15 sec. The pictures were presented in a random order in one run lasting 9 min and 30 sec.

Stimuli were presented using a Linux laptop computer with in-house stimulus delivery software. They were projected using an Epson MP 7200 LCD projector onto a screen placed at the foot of the MRI scanner bed and were viewed using a mirror mounted on the head coil. Each stimulus presentation lasted 800 msec (two volume acquisitions). Data suggest that a quick glimpse of emotionally relevant stimuli appears sufficient to tune the brain for selective perceptual processing (Schupp et al 2004). Participants were instructed to attend to the pictures, but no motor response was required. Participants were asked to rate their mood on a five point scale using the Positive and Negative Affect Scales (Watson et al 1988) at baseline (prescan) and immediately following each scan (postscan).

fMRI acquisition

Imaging was performed using a 3 T General Electric MRI scanner (General Electric, Milwaukee, WI) and a standard quadrature head coil. In-plane resolution was 3.75 x 3.75 mm. Functional scans were acquired using a $T2^*$-sensitive echoplanar sequence with a repetition time (TR) of 400 msec, echo time (TE) of 40 msec, and flip equal to 30°. We collected five 5.0 mm contiguous axial slices drawn from the base of the orbitofrontal cortex upward to the level approximately at the base of the mid-corpus callosum, which allowed us to image most of the temporal and ventral frontal lobe. Five slices was the maximum number we could collect at a TR of 400 msec. This short TR was chosen because it allowed for selective filtering of noise due to the cardiac cycle (Rio 2006). To allow for signal stabilization, 30 acquisitions were obtained before task onset. A total of 1430 volumes were collected. Structural scans were acquired using a

T1-weighted MP-RAGE (magnetization-prepared rapid gradient echo) sequence (TR, 100 msec; TE, 7 msec; flip, 90°), which facilitated localization and co-registration of functional data.

FMRI analysis

Analyses focused on changes in BOLD signal contrast (hereafter, activation) that occurred as the participants viewed the positive and negative pictures and the beverage cues. Analyses were conducted using Analysis of Functional Neural Images (AFNI) software (Cox 1996). Echoplanar image volumes were preprocessed as follows: (1) voxel time series were interpolated to correct for non-simultaneous slice acquisition within each volume (using sinc interpolation and the most inferior slice as a reference), and (2) volumes were corrected for motion in three-dimensional space. Motion-correction estimates indicated that no participant's head moved >1.0 mm in any dimension from one volume acquisition to the next. We imposed a 6 mm full-width half-maximum (FWHM) smoothing kernel in the spatial domain. (3) We created a mask so that all of the background values outside of the brain were set to zero, so that we could calculate the percentage signal change in each voxel.

This analysis was conducted in two stages. First, statistical maps were generated for each individual separately by linear contrasts of combinations of the four regressors of interest. The four regressors of interest were: (1) positive IAPS image with alcohol cue, (2) positive IAPS image with non-alcohol beverage cue, (3) negative IAPS image with alcohol cue, and (4) negative IAPS image with non-alcohol beverage cue. Preprocessed time series data for each individual were then analyzed by multiple regression, which allowed co-variation of variables related to head motion and scanning run. The regression model consisted of the four orthogonal regressors of interest and six regressors of no interest modeling residual motion after volume registration. Regressors of interest were convolved with a gamma-variate function that modeled a prototypical hemodynamic response before inclusion in the regression model (Cohen 1997). Idealized signal time courses were time-locked to image onset. Anatomical maps of *t* statistics representing each of these regressors of interest were spatially normalized by warping to Talairach space and

combined into a group map. We applied a family-wise error rate correction (using a Monte Carlo simulation) to rule out false positives. Only clusters larger than 6 contiguous voxels (individual voxel threshold probability = 0.005) were considered significant.

Second, we calculated a statistical map of the differences in activation within-groups for each contrast (negative vs positive IAPS image with non-alcoholic beverage cue, negative vs positive IAPS image with alcohol cue, alcohol vs non-alcohol cue with negative IAPS image, and alcohol vs non-alcohol cue with positive IAPS image) by performing voxel-wise *t* tests of the event-related β coefficients calculated from the general linear model (using inputs of the regression model). We also ran between-subjects *t* tests to test for differences between alcoholics and controls for each of the four inputs. These single factor ANOVAs compared normalized event-related β weights that were subsequently characterized by assessment of actual BOLD signal changes in regions of interest that were highly activated during the task.

Post hoc analysis: Volume of Interest (VOI).

Differences between groups in event-related signal changes in specific regions were characterized with VOI analyses, in which time series signal data from the same brain coordinates in both groups were analyzed. The three regions we selected were the right amygdala (coordinates: 25, -12, -9), the right parahippocampal gyrus (32, -9, -11), and the right lingual gyrus (22, -77, -3). We selected these regions because they had previously been implicated in emotional image processing (Vuilleumier 2005), or they were robustly activated in this task. The VOIs were drawn as three dimensional spheres with a radius of 5 mm. Signal data were extracted from the time series as follows: (1) signal at each voxel was converted to a (percentage) deviation from the mean for that voxel across the entire time series, (2) signal was averaged by stimulus type and spatially translated into Talairach space, and (3) impulse response functions (IRFs) were estimated by generating a 20 TR (or 8.0 second) time course following the presentation of each stimulus category, which estimated 20 coefficients, covering lags from 0 to 8.0 sec after stimulus presentation. We generated mean IRFs for each of the four conditions, and averaged the percent

signal change from 2 sec to 8 sec from stimulus onset to get a mean response magnitude for each stimulus type. We used a repeated measures MANOVA to examine the independent variable of group diagnosis (alcoholic or control), on the dependent variable of mean percent signal change in each of the four stimulus conditions (package JMP-SAS; SAS Institute, Cary, North Carolina).

Anxiety Ratings.

Alcohol-dependent patients were given the Comprehensive Psychopathological Rating Scale (Svanborg and Asberg 1994) within five days of their fMRI scan.

2.3. Results

Self-report affect ratings. There were no significant differences in positive affect (PA) between controls and alcoholics before or after the scan. Alcoholics scored higher on negative affect (NA) than controls before the scan ($p = 0.013$) but showed no difference in NA after the scan. Neither group showed a significant difference in PA or NA between pre-scan and post-scan.

Brain activation analysis: Within-Group Comparisons.

Brain regions that differed significantly between conditions within the alcoholics are depicted in **fig. 2.2.** There were no significant differences between conditions detected in controls (data not shown).

Comparison 1: Negative vs Positive IAPS Images Paired with Non-Alcohol Beverage Cues

This direct linear comparison provided an examination of the response to the positive and negative images in the absence of alcohol cues (i.e., in the presence of non-alcoholic beverage cues). Brain regions that differed significantly are reported in **table 2.2**. Alcoholics showed more activation to negative images than positive images in 10 distinct clusters. The largest clusters were found in the bilateral insula, inferior frontal gyri (IFG), lingual gyri, parahippocampal gyri, and right medial temporal gyrus (MTG). Control subjects did not show increased activation to negative relative to positive images in any region.

Comparison 2: Non-Alcohol vs Alcohol Beverage Cues Paired with Negative Images

This comparison, reported in **table 2.3**, directly tested how alcohol cues modulated the response to negative images in alcoholics and controls. Alcoholic patients had higher activation to the negative images paired with the non-alcohol cues compared to those paired with alcoholic beverage cues in 8 clusters. The largest clusters to show increased activation were in the left inferior temporal gyrus (ITG), bilateral lingual gyri, bilateral fusiform gyri, and bilateral medial occipital gyri. Control participants did not show any differences in activation to negative images with the non-alcohol compared to alcohol cues.

Comparison 3: Alcohol vs Non-alcohol Beverage Cues Paired with Positive Images

This comparison tested whether the alcohol cues modulated positive emotional processing. Alcoholics showed higher activation to positive images paired with the alcohol cues relative to non-alcohol cues in the bilateral IFG, and decreased activation in the bilateral inferior occipital gyrus. Control participants showed higher activation to the positive images with alcohol cues relative to non-alcoholic cues in the right parahippocampal gyrus and left superior temporal gyrus.

Comparison 4: Positive vs Negative Images Paired with Alcohol Beverage Cues

This comparison tested how the alcohol cues themselves affected valence-specific response to positive and negative images. Alcoholics showed higher activation to the positive than to negative IAPS images paired with alcohol cues in 8 clusters, reported in **table 2.4**. The largest clusters were found in the bilateral medial occipital gyri (MOG), lingual gyri, inferior occipital gyrus, right fusiform gyrus, and left parahippocampal gyrus. Controls showed higher activation to positive than to negative images in the left inferior occipital gyrus (IOG).

Brain activation analysis: Between-Group Comparisons

Brain regions that differed significantly across conditions between alcoholics and controls are reported in **table 2.5**, and depicted in **fig. 2.3**.

Negative Images with Non-alcoholic Beverage Cues.

This comparison examined how response to negative images, in the absence of alcohol cues, differed between alcoholics and controls. Alcoholics demonstrated greater activation than controls in 6 clusters. The largest clusters were found in the left hippocampus, bilateral parahippocampal gyri, and right lingual gyrus. There were no clusters in which the alcoholics had less activation than controls.

Negative Images with Alcohol Beverage Cues.

In this comparison, we examined how alcoholics compared to controls when viewing negative images in the presence of alcohol cues. In contrast to the large differences between alcoholics and controls when their brain responses to negative images with non-alcoholic beverage cues were compared, alcoholics and controls differed much less when the negative images were paired with alcohol cues. Alcoholics showed more activation only in the right MTG. Controls displayed higher activation than alcoholics in the left lingual gyrus and the fusiform gyrus.

Positive Image with Neutral Beverage Cue.

In this comparison, alcoholics showed less activation than controls in 8 clusters, including the right MTG, right IFG, left insula, and right hypothalamus. There were no areas where alcoholics had more activation than controls.

Positive Image with Alcohol Beverage Cue.

When viewing positive images with alcohol cues, alcoholics showed less activation than controls in the right hippocampus. There were no areas where alcoholics had more activation than controls.

VOI Analysis.

In each analysis, we ran a mixed-model ANOVA, using group (control or alcoholic) as a between-subject factor and condition (negative IAPS – alcohol beverage, negative IAPS- neutral

beverage, positive IAPS- alcohol beverage, and positive IAPS – neutral beverage) as the within-subjects factor. In this model, the independent variable was peak percent signal change in each of the three VOIs.

(1) *Right amygdala:* We found a significant group effect in the amygdala ($F(3,23) = 27.08$, $p < 0.0001$). We did not find a significant within-subject effect of condition, indicating that the alcoholics showed an increased response in the amygdala relative to controls in each of the conditions.

(2) *Right parahippocampal gyrus:* In the right parahippocampal gryus, we found an effect of group ($F(3,23) = 4.958$, $p = 0.037$) and condition ($F(3, 23) = 3.51$, $p = 0.035$), but no significant interaction. Alcoholics showed an increased response relative to controls, but the groups did not significantly differ across conditions.

(3) *Right lingual gyrus:* We did not find any significant effects in the lingual gyrus.

Correlation between Anxiety and Percent Signal Change in the Right Parahippocampal Gyrus (Alcoholic patients only)

In the negative IAPS – non-alcohol beverage condition, anxiety self-ratings significantly correlated with activation in the right parahippocampal gyrus ($r^2 = 0.44$; $p = 0.02$) (**fig. 2.4**). In the negative alcohol condition, anxiety did not predict signal change. In the positive non-alcohol condition, there was a significant effect of anxiety ($r^2 = 0.51$; $p = 0.01$). Anxiety did not correlate with signal change in the positive alcohol condition. Correlations were not significant in the amygdala or the lingual gyrus.

2.4. Discussion

There are three main findings of this study. First, in the absence of the alcohol cues (positive and negative IAPS – neutral beverage conditions), alcohol-dependent patients showed significantly more activation to negative than to positive images. The greater activation to threatening images was located in the ventral, object-related visual processing stream and in the insular cortex. Alcoholics also showed significantly greater activation than control participants to negative images themselves without adjusting for response to positive images. Second, when the images were presented along with the alcohol cues, there was a decrease in the difference in activation between the emotionally positive and negative images among the alcoholics, and a decrease in the difference in response to the negative images between controls and alcoholics. The presence of alcohol images reduced the increased activation to emotionally negative images we observed among alcoholics in the hippocampus, parahippocampal and lingual gyri, so that the alcoholics' activation to threatening stimuli was more similar to the activation observed among non-alcoholics. Third, in the positive and negative IAPS - neutral beverage conditions, anxiety ratings among the alcoholics significantly predicted activation in the right parahippocampal gyrus, but self-reported anxiety did not predict activation when the alcohol cues were presented with positive or negative images.

Increased Activation to Negative Images in Alcoholics.

Many studies have shown that visual processing is enhanced by emotion (see Vuilleumier 2005 for review). Imaging studies of healthy controls have shown that both positive and negative emotional images elicit robust activation in both early and late visual processing areas (Lane et al 1999). In this study, control participants did not demonstrate significant differences between activation to negative and positive images, which is consistent with other studies that have failed

to show differences in activation between pleasant and unpleasant stimuli in healthy controls (Klein et al 2003).

Patients with psychiatric disorders, however, show increased activity relative to controls in response to negative stimuli. For example, patients with major depressive disorder show increased activity in the right fusiform gyrus, ventral striatum, and left parahippocampal gyrus to sad faces compared to healthy controls (Surguladze et al 2005). Depressed patients also show increased activation in cortical and limbic regions compared to healthy controls when viewing negative emotional slides (Anand et al 2005). We found increased activation in alcoholics to negative images in many cortical regions along the ventral visual stream, including the hippocampus, parahippocampal gyrus, fusiform gyrus, and lingual gyrus. Neurons in the amygdala have direct monosynaptic projections to all occipital and temporal levels in the visual system, which suggests that the amygdala can modulate processing at all stages of the visual stream (Amaral et al 2003). When we used a VOI approach to examine activation in the amygdala we found that alcoholics had a higher level of BOLD in amygdala than the non-alcoholics, irrespective of the image pair presented. This suggests that among alcoholics the amygdala is more responsive to meaningful visual images in general. This is consistent with the observation that, in addition to modulating fear processing, the amygdala is also involved in the processing of a wide array of biologically relevant stimuli (Sander et al 2003).

Modulation of Brain Response to Emotional Images by Alcohol Cues.

There are two possible explanations for the attenuated response to the negative images in the presence of alcohol cues we observed among the alcoholics. First, this modulation could be a function of cognitive interference, in which processing of one stimulus impedes simultaneous processing of another stimulus (van den Heuvel et al 2005). Cognitive interference has historically been demonstrated using the Stroop task, in which a subject is presented with a word which names a color, and is instructed to name the *color* of the word. The emotional analog of the

Stroop task consists of presenting colored words with emotionally charged meaning, or with meaning relevant to one's own concerns (i.e. presenting the word "crowd" to a social phobic). The Stroop task has been used to investigate various disorders, such as anxiety, panic disorder, obsessive compulsive disorder, post-traumatic stress disorder, social phobia, and spider phobia, and this has consistently yielded results showing increased interference to threatening words in patients compared to healthy controls (see van den Heuvel et al 2005 for review). While the Stroop task intentionally divides the subject's attention, Kastner et al (1998) found that by simply presenting multiple stimuli in the visual field, activation to each stimulus will interact in a suppressive fashion. Directing attention to one stimulus counteracts the suppressive influence of other stimuli, and therefore, if one stimulus is more salient than another, competition will decrease and activation to the dominant stimulus will be greater (Kastner et al 1998). According to this logic, if a complex, emotional image is presented with a neutral beverage cue, attention will be directed to the emotional image, and suppression will not occur. However, if alcoholic patients are forced to split attention between a salient drug cue and an equally salient image, the decreased responsiveness to the negative images could have been to due cognitive interference between the two images. Since the selected IAPS images were rated highly in both arousal and valence (either positively or negatively), and all contained fairly complex, emotional scenes, control participants likely attended to the IAPS images and not to the beverage cues. This may explain why we did not observe suppression in activation in these subjects.

However, unlike other studies that compared a single stimulus to two competing stimuli in the visual field, this study compared pairs of stimuli that may have acquired different levels of salience as a function of the internal states of the groups. Among alcoholics, beverage cues may have become "attention grabbing" stimuli (Robinson and Berridge 1993) and may have competed with the negative images for limited attentional resources in a way that would not occur among control participants. Many studies have shown that appetitive/pleasant stimuli also capture attention (see Franken 2003 for review), and it is possible that during the simultaneous

presentation of the IAPS images with the alcohol cues, the alcoholic patients more so than the controls attended to the alcohol cues.

Although cognitive interference is a possible reason for the attenuated brain activity to the negative images, if this were the only explanation, then the alcohol cues would have equally decreased the neural activity to the positive images. The alcohol cues did not, however, have a robust effect on the neural activation to the positive images. An alternative explanation for the reduction in brain response to the negative images in particular in the presence of an alcohol cue is that the cue itself may have become anxiolytic to the alcoholic patients. Alcoholic patients may associate alcohol cues with the positive, rewarding properties of alcohol. The cues may have modulated the cortical networks involved in the processing of emotional stimuli by eliciting a conditioned response in the alcoholics, but not in the controls, which in turn may have blunted activation to negative images.

It should be noted that previous studies have reported that alcohol-related cues activate nucleus accumbens and ventral tegmental area (Kareken et al 2004), insula, prefrontal cortex and ventral putamen (Olbrich et al 2006) and medial prefrontal cortices, visual cortices, and anterior cingulate (Grusser et al 2004). In the current study, we did not observe greater activation in these regions to the alcohol cues relative to the neutral beverage cues, perhaps because of the simultaneous presentation of the cues with the IAPS images. It is possible that the IAPS images were more highly-arousing than the alcohol cues, and therefore, the brain activity observed was primarily in response to the emotional images.

Correlation of Activation of the Right Parahippocampal Gyrus with Anxiety Scores in Non-Alcohol but not Alcohol Cue Condition.

We found that the alcohol cues modulated brain activity in several areas, but consistently in the parahippocampal gyrus. The parahippocampal gyrus has multiple direct connections with both the hippocampus and the amygdala, and many studies implicate this region in the processing

of both visual-spatial information (Burgess et al 2002; Henson 2005; Sommer et al 2005) and intense emotional images (Surguladze et al 2006). In schizophrenic patients, there is a positive association between the right amygdala and the right parahippocampal gyrus responses to fearful faces and severity symptoms, suggesting that overactive parahippocampal regions may be associated with impaired context/emotional appraisal (Surguladze et al 2005). The hippocampus and the parahippocampal gyrus are often damaged with prolonged alcoholism (see White et al 2000 for review), and this may relate to the increased activity in response to emotional images in these regions.

The correlation between parahippocampal response to negative and positive emotional images and anxiety scores suggests that anxious patients may have a greater response to any highly arousing emotional image. When the alcohol cue was presented with the image, the correlation was no longer significant. Fox et al demonstrated that highly anxious individuals were unable to rapidly disengage visual attention away from emotional stimuli (Fox 2001) and it is possible that anxious alcoholics may require a particularly salient competing stimulus, such as an alcohol cue, in order to shift attention. The right parahippocampal gyrus may be involved in the modulation of attention to emotional images.

Limitations and future directions

The major limitation of this study is the absence of eye tracking data that could determine the location of gaze of the participants during the simultaneous presentation of the IAPS image and the beverage cue. Many studies have demonstrated attentional biases to drug cues by drug abusers, and we therefore assume the same biases in our participants, but we have no way of determining if alcoholic patients attended to the alcohol cues longer than did the control participants. The simultaneous presentation of the IAPS images and the alcohol cues makes interpretation of results difficult. Future research can incorporate eye-tracking measures to enable relation between cue-based differences in brain activation and concomitant changes in

attention allocation. In addition, to investigate how alcohol cues affect emotional processing, different modalities can be utilized (i.e. visual affective images and alcoholic odor cues).

In summary, this study demonstrates a reduction in brain response to negative images in the presence of drug cues among drug users. Since alcoholics report that they use alcohol to blunt the effects of painful, threatening, or fearful emotions (Woody 1992), our ability to measure this phenomena in the brain may be of considerable value in the development of medications for the treatment of alcoholism. It should be possible not only to measure anxiolytic actions of putative treatments but also to determine if a treatment can reduce the ability of alcohol cues themselves to blunt the expression of brain states underlying fear.

Table 2.1. Demographic Characteristics of Study Groups

	Alcoholics (n = 12)	Controls (n = 12)
Age, mean (SD)	41.83 (8.39)	38.08 (6.97)
Years of Education	13.79 (2.49)	17.41 (1.88)
Average # drinking days/month	27.25 (5.66)	3.09 (2.25)
Average # drinks/drinking day	15.5 (9.32)	2.27 (1.62)
# comorbid drug abusers*	8/12	0
# patients with Axis I disorders	10/12	0
# Mood Disorder	4/12	0
# Anxiety Disorder	4/12	0
# patients with Axis II disorders	3/12	0

All categories differ significantly between groups ($p < 0.05$) except for age.

* Drugs of abuse included cocaine (7 patients), cannabis (6), sedatives (2), opioids (2), amphetamine (1), and hallucinogens (1). All patients reported alcohol dependence as their primary complaint.

Table 2.2. Negative vs Positive IAPS Images Paired with Non-Alcohol Beverage Cues (Negative Non-alcohol > Positive Non-alcohol)

Group	Brain Region	Talairach coordinates *x*	*y*	*z*	Volume (mm^3)	*t*-score	*p* (uncorrected)
Alcoholics	Left Insula	-41	-23	-8	5231	4.72	< 0.001
	Right Inferior Frontal Gyrus	41	19	-8	4556	4.47	< 0.001
	Left Lingual Gyrus	-19	-45	-2	3122	5.39	< 0.001
	Right Medial Temporal Gyrus	45	-49	4	2700	5.54	< 0.001
	Right Insula	41	-15	-2	2616	3.56	< 0.005
	Right Parahippocampal Gyrus	23	-34	-2	2194	4.66	< 0.001
	Right Lingual Gyrus	19	-71	4	2194	4.19	< 0.005
	Left Inferior Frontal Gyrus	-34	23	-8	1856	3.50	< 0.005
	Left Parahippocampal Gyrus	-38	-34	-14	1688	4.75	< 0.001
	Right Thalamus	8	-8	-2	1097	3.83	< 0.005
Controls	No Clusters Detected						

Threshold is set at $p < 0.005$ uncorrected and a voxel threshold of at least 6 active voxels which yields a family-wise error rate correction of $p < 0.05$.

Table 2.3. Non-Alcohol vs Alcohol Cues Paired with Negative IAPS Images (Negative Non-alcohol > Negative Alcohol)

Group	Brain Region	Talairach coordinates			Volume (mm^3)	*t*-score	*p* (uncorrected)
		x	*y*	*z*			
Alcoholics	Left Inferior Temporal Gyrus	-45	-64	-2	4050	5.55	< 0.001
	Right Lingual Gyrus	23	-75	-2	3712	6.51	< 0.0001
	Left Fusiform Gyrus	-45	-56	-8	3628	4.35	< 0.005
	Right Fusiform Gyrus	45	-53	-8	2025	4.33	< 0.005
	Left Lingual Gyrus	-19	-45	-2	1941	4.17	< 0.005
	Left Medial Occipital Gyrus	-23	-86	4	1856	4.58	< 0.001
	Right Medial Occipital Gyrus	38	-75	4	1688	3.44	< 0.005
	Right Thalamus	11	-30	-2	759	3.56	< 0.005
Controls	No clusters detected						

Threshold is set at $p < 0.005$ uncorrected and a voxel threshold of at least 6 active voxels which yields a family-wise error rate correction of $p < 0.05$.

Table 2.4. Positive vs Negative IAPS Images Paired with Alcohol Cues (Positive Alcohol > Negative Alcohol)

Group	Brain Region	Talairach coordinates			Volume		*p*
		x	*y*	*z*	(mm^3)	*t*-score	(uncorrected)
Alcoholics	Right Middle Occipital Gyrus	30	-83	-8	1688	4.96	< 0.001
	Left Lingual Gyrus	-26	-79	-2	1688	4.71	< 0.001
	Right Inferior Occipital Gyrus	34	-71	-2	1603	4.54	< 0.001
	Right Fusiform Gyrus	38	-53	-14	1519	3.44	< 0.005
	Left Parahippocampal Gyrus	-38	-38	-8	1181	3.72	< 0.005
	Right Inferior Temporal Gyrus	45	-45	-8	928	3.64	< 0.005
	Right Lingual Gyrus	11	-79	-2	844	5.20	< 0.001
	Left Medial Occipital Gyrus	-41	-68	-2	759	4.20	< 0.005
Controls	Left Medial Occipital Gyrus	-30	-86	4	1097	3.56	< 0.005

Threshold is set at $p < 0.005$ uncorrected and a voxel threshold of at least 6 active voxels which yields a family-wise error rate correction of $p < 0.05$.

Table 2.5. Brain regions that differ significantly between alcoholics and controls in each [condition.

Condition	Comparison	Brain Region	Talairach coordinates *x*	*y*	*z*	Volume (mm^3)	*t*-score	*p* *(uncorrected)*
Negative	Alcoholics >							
Non-Alcohol	Controls	Left Hippocampus	-34	-30	-8	1856	3.84	< 0.001
		Right Lingual Gyrus	19	-79	-2	1350	3.69	< 0.005
		Left Parahippocampal Gyrus	-38	-34	-14	759	3.58	< 0.005
		Right Thalamus	19	-26	4	675	3.12	< 0.005
		Right Lingual Gyrus	23	-79	-8	506	3.20	< 0.005
		Right Inferior Occipital Gyrus	34	-79	-2	506	3.43	< 0.005
	Controls >							
	Alcoholics	No clusters detected						
Negative	Alcoholics >							
Alcohol	Controls	Right Middle Temporal Gyrus	64	-34	-8	506	3.41	< 0.005
	Controls >							
	Alcoholics	Left Lingual Gyrus	-8	-75	-2	928	3.54	< 0.005
		Right Fusiform Gyrus	41	-41	-14	506	3.14	< 0.005
Positive	Alcoholics >							
Non-Alcohol	Controls	No clusters detected						
	Controls >							
	Alcoholics	Right Medial Temporal Gyrus	60	-30	-2	1519	3.45	< 0.005
		Right Inferior Frontal Gyrus	30	19	-14	844	3.32	< 0.005
		Left Insula	-41	-15	-8	675	3.48	< 0.005
		Right Hypothalamus	11	-4	-2	591	3.32	< 0.005
		Left Inferior Frontal Gyrus	-23	15	-14	506	4.25	< 0.001
		Left Superior Temporal Gyrus	-41	0	-14	506	3.37	< 0.005
		Right Medial Temporal Gyrus	53	-38	-2	506	3.22	< 0.005
		Right Middle Temporal Gyrus	8	-11	-8	506	3.26	< 0.005
Positive	Alcoholics >							
Alcohol	Controls	No clusters detected						
	Controls >							
	Alcoholics	Right Hippocampus	26	-26	-14	591	3.32	< 0.005

Threshold is set at $p < 0.005$ uncorrected and a voxel threshold of at least 6 active voxels which yields a family-wise error rate correction of $p < 0.05$.

Fig 2.1. Examples the four classes of stimuli. Half of the beverage cues were presented to the left of the IAPS image, and half were presented on the right.

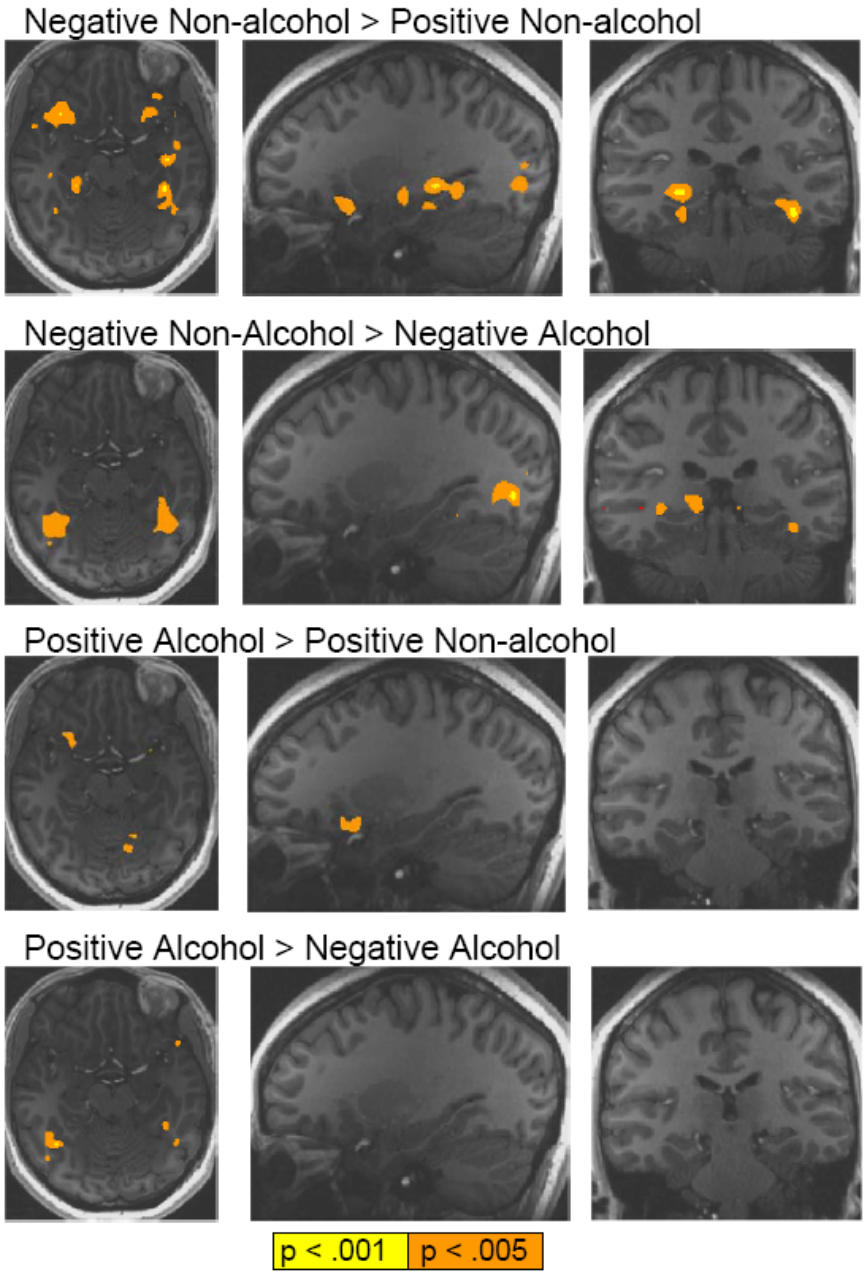


Fig 2.2. Linear contrasts of regional brain activation in alcoholics. Group statistical maps are superimposed upon a T1 structural image in Talairach space. Clusters ≥ 6 contiguous voxels ($p < 0.05$ corrected) are considered significant. The color scale reflects the p-value.

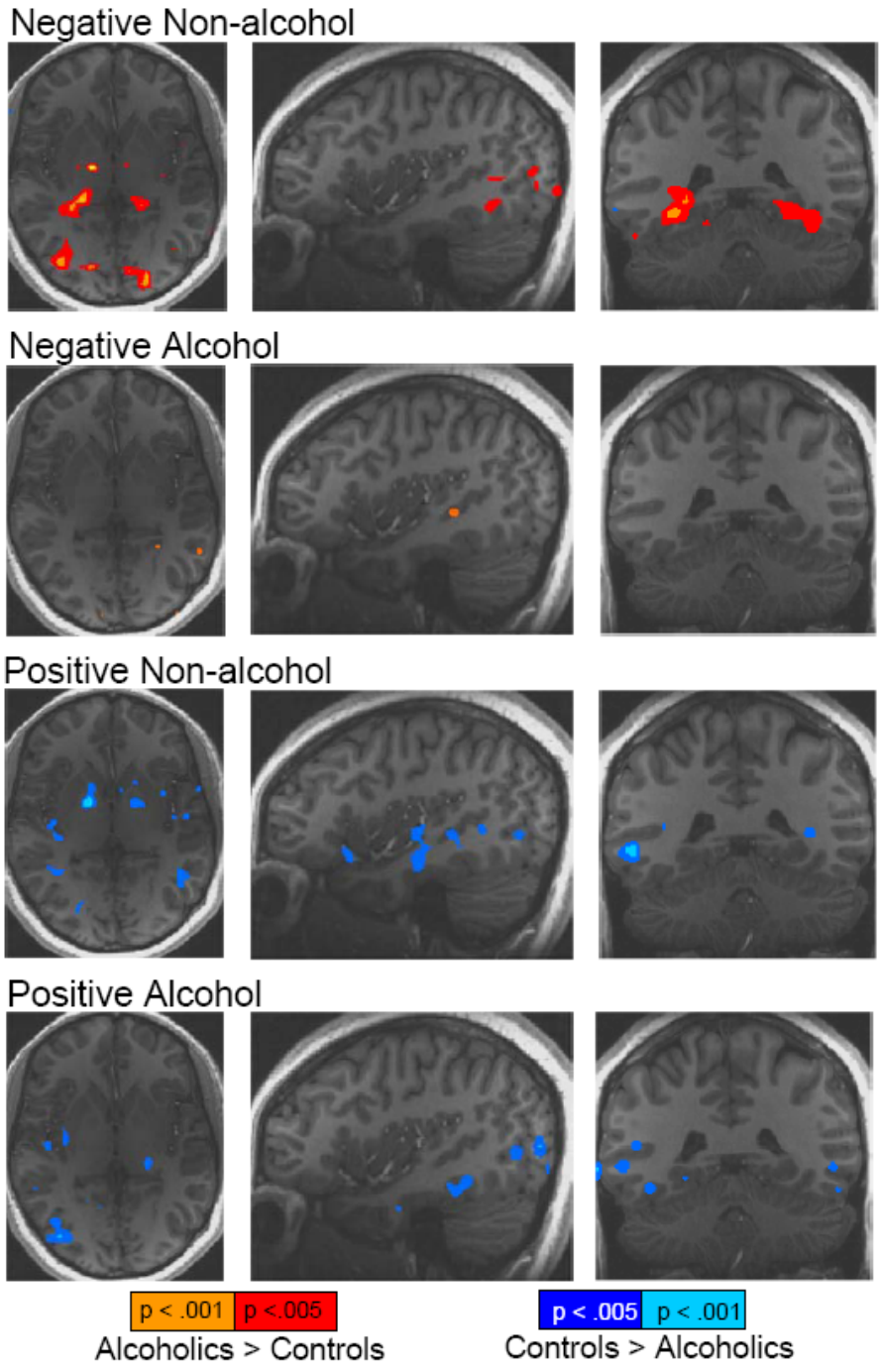


Fig 2.3. Between-group differences in regional brain activation in response to each condition. Yellow-orange regions indicate more activation in the alcoholics, while blue regions indicate more activation in controls. Group statistical maps are superimposed upon a T1 structural image in Talairach space. *T*-tests were conducted between groups for each condition. The color scale reflects the *p*-value.

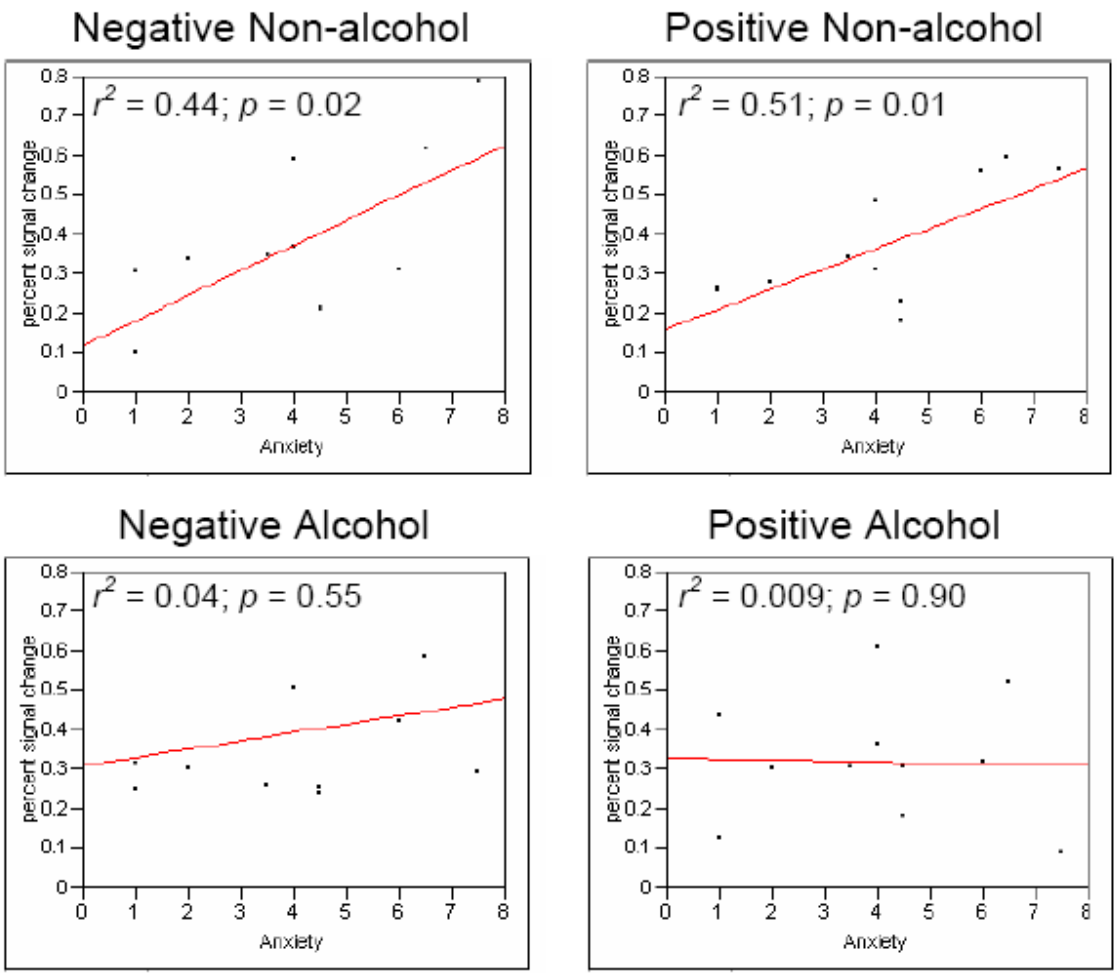


Fig 2.4. Positive correlations between percent signal change in the right parahippocampal gyrus and anxiety scores in alcohol-dependent patients. Correlations were significant the negative non-alcohol (r^2 = 0.44; p = 0.03) and positive non-alcohol (r^2 = 0.51; p = 0.01) condition, but not in the negative alcohol (r^2 = 0.04; p = 0.55) or positive alcohol (r^2 = 0.009; p = 0.9) condition.

Chapter 3:

Modulation of Brain Response to Emotional Images by Treatment with an Anxiolytic Drug

The second experiment in this thesis extends the findings of the previous experiment by investigating whether the increased sensitivity to negative images and decreased sensitivity to positive images observed in alcoholic patients can be modulated pharmacologically. To address this question, patients were either treated with an anxiolytic drug, LY686017, or a placebo control drug, and the experiment outlined in Chapter 2 was repeated. If the differential brain response to negative and positive images is related to higher anxiety in alcoholics, then the patients treated with the drug are expected to exhibit a reduced brain response to negative images and an enhanced brain response to positive images relative to non-treated patients. If LY686017 also affects alcohol craving, then we expect to see decreased activation to alcohol cues in patients on active drug relative to patients on a placebo treatment. [4]

[4] Some tables and figures in this chapter are taken from a published manuscript: Neurokinin 1 receptor antagonism as a possible therapy for alcoholism. George, D.*, **Gilman, J**.*, Hersh, J.*, Thorsell, A.*, Herion, D., Geyer, C., Peng X., Rawlings R., Gehlert, D., Tauscher J., Hommer, D., and Heilig, M. (*these authors contributed equally). *Science,* Mar 14; 319(5869):1536-9.

3.1. Introduction

As shown in the previous chapter, and consistent with Koob's theory of addiction (Koob 1992), alcohol-dependent patients demonstrate increased brain activation to negative images in several brain areas that have been implicated in the processing of emotion, including the insula and the parahippocampal gyrus, and well as a decreased response to positive images. Few studies have examined the neural response to positive images, but the increased response to negative images has been observed in other psychiatric populations (i.e. patients with depression, phobias, anxiety disorders, and panic disorders (Bradley et al 1997; Lang and Sarmiento 2004; Mathews et al 1996; Spector et al 2003)). This suggests that the increased responsiveness to fear-inducing stimuli may be related to the increased anxiety observed in our alcoholic population.

The previous study also demonstrated that although alcoholic patients exhibit increased brain responsive to negative images, this response can be modulated by an alcohol cue which may be a conditioned anxiolytic stimulus. In the current study, we investigate whether neural activation can also be modulated pharmacologically by treatment with an anxiolytic drug, LY686017 (Amegadzie 2003). Furthermore, we investigate whether this drug also influences the neural response to visual alcohol cues, which may be indicative of alcohol craving.

Pharmacological fMRI, the combination of MRI with the study of drugs (Wise and Tracey 2006), is a new and challenging area of research. This technique aims to measure the modulation of regional brain activity by centrally active drugs. Traditionally, researchers have used positron emission tomography (PET) when studying drugs that affect the brain, because this technique allows for the direct measurement of receptor occupancy of a drug. PET confers information about whether the drug reaches its target, how much of the drug is absorbed into the tissue, how the drug is eliminated from the tissue, and if the drug is efficacious (Gupta et al 2002). However, PET studies are more relevant for preclinical assessment of psychiatric drugs than for assessing their behavioral or psychological effects. In addition, because PET relies on

the injection of radioactive tracers, experiments cannot be repeated on the same subjects. To measure the efficacy of psychiatric drugs in humans, researchers and clinicians have traditionally been limited to behavioral observations and self-report questionnaires.

Pharmacological fMRI has many advantages over behavior and self-report. This technique can accurately assess the effects of acute drug administration on neuronal activity, the effect of the drug on task-related neuronal activity, and the long-term functional changes associated with chronic drug use (Salmeron and Stein 2002). In addition, fMRI can be combined with behavioral measures that can identify not only brain areas affected by the compound, but also brain regions associated with behavioral aspects of the drug response. For example, Breiter and colleagues used fMRI to study the neural response to intravenous cocaine in cocaine-addicted patients (Breiter et al 1997) . In this experiment, subjects were asked to provide self ratings every minute of "high," "rush," "craving," and "low." Ratings were used to cross-correlate with the signal, and the researchers found that "rush" was associated with activity in the ventral tegmental area, caudate, and prefrontal cortex, while "craving" was associated with activity in the nucleus accumbens. fMRI can measure the interaction between subjective feelings/task performance, BOLD activity, and drug concentration, which allows researchers to better understand how the drug relates to neural activity, emotion, and performance effects (Wise and Tracey 2006).

The current experiment is one of the first pharmacological fMRI studies in the alcoholism field. Several studies suggest that the use of highly arousing emotional images to elicit a neural response in anxious alcoholics will be a useful probe to assess the effects of LY686017. Alcohol dependence is highly co-morbid with mood- and anxiety disorders (Grant et al 2004; Hesselbrock et al 1985), a group of disorders characterized by negative affect, and even in the absence of mood disorders, alcohol use per se induces negative affect during both acute and protracted withdrawal. Because of this, reduction of negative affect is a key objective for the development of novel pharmacological treatments for alcohol dependence. More specifically, stress-induced activation of the amygdala complex is key to mediating behavioral stress responses, and

preclinical data indicate that inhibition of this response might be beneficial in reducing voluntary ethanol drinking (Moller et al 1997).

Substance P (SP), an 11 amino acid peptide belonging to the neurokinin family, and its preferred NK1 receptor, are highly expressed in brain areas involved in stress-related behaviors (Hargreaves 2002). In addition to its possible actions related to stress, NK1 may also be involved in reward (Gadd et al 2003). In mice, stressors lead to the release of SP in the amygdala, while deletion or selective blockade of NK1 receptors blocks associated behavioral responses. Furthermore, in mice, genetic deletion of NK1 receptors causes a loss of conditioned place preference for opiates, as well as opiate-self administration (Murtra et al 2000; Ripley et al 2002), and recent data indicate marked reduction of alcohol drinking in NK1 receptor null-mutants (Pastor 2005).

The discovery of anti-stress actions produced by NK1 antagonism has guided the clinical development of an NK1 antagonist. LY686017 (Amegadzie AK 2003) is a high-affinity, selective, non-peptide, orally available NK1 antagonist. We hypothesize that blockade of NK1 receptors in humans may modulate stress- and reward-related processes that are important in excessive alcohol use and relapse. We expect to observe a suppression of craving for alcohol and a reduction of neural activity in response to negative, fear-inducing stimuli in alcohol-dependent patients treated with LY686017.

3.2. Methods

Recruitment and Subjects

Participants (25 in each arm of the study) were recruited among patients admitted to the NIAAA inpatient care unit at the NIH Clinical Research Center (CRC) in Bethesda, MD, under a general protocol for assessment and treatment of alcohol use disorders. Medically assisted withdrawal was completed if needed, following which subjects were evaluated for eligibility. Subjects were eligible if they were 21-65 years of age, had a diagnosis of alcohol dependence based on structured diagnostic interview (First 1996), reported alcohol problems as their primary complaint, had used alcohol within the last month, and scored > 39 on the Spielberger Trait Anxiety Inventory (STAI) (Spielberger CD 1983). Subjects were excluded if they had complicated medical or psychiatric problems, had received psychotropic medication other than withdrawal treatment in the last 4 weeks, were court ordered to treatment, or were likely to become incarcerated during the course of the study. The study was approved by the Institutional Review Board (IRB), and informed consent was obtained according to IRB approved procedures.

Overall design

Subjects started in the study after a minimum of 2 days without significant withdrawal symptoms. Following inclusion, all subjects began a 1 week single-blind placebo lead-in period. Subjects were then randomized to receive active drug or placebo during weeks 2 through 4. LY686017 was given as a 50 mg once-daily oral dose. Ratings of spontaneous cravings, clinician's global impression, and general psychopathology were obtained throughout the 4 week study as described below. During week 4, an fMRI scan was obtained to assess brain responses to emotional stimuli.

Assessment instruments and rating scales

To assess spontaneous alcohol cravings over time on the inpatient unit, the self-report based Alcohol Urge Questionnaire (AUQ) (Bohn et al 1995) was collected twice weekly in the

evening. For global assessment of alcohol related pathology over time, the clinician rated clinical global impression (CGI) was obtained weekly (Guy 1970). The self-report version of the Comprehensive Psychopathological Rating Scale, Self-Affective (CPRS-SA) (Svanborg and Asberg 1994) was collected twice weekly to obtain measures of general psychopathology. This instrument provides subscales for depressive and anxious symptoms.

fMRI study

To evaluate the ability of NK1 antagonism to modulate brain responses to emotional stimuli in alcoholics, blood oxygen-level dependent (BOLD) activity was measured during presentation of stimuli composed of negative and positive emotional stimuli from the International Affective Picture System (IAPS) (Lang 1995) and alcoholic or neutral beverage cues. This task had been used in our lab to show greater BOLD response to fearful images among alcoholics and reduction of this response by alcohol beverage images (Gilman 2008).

Visual stimulation and task: Fifty-five high-arousal negative pictures and 55 high-arousal positive pictures from the IAPS were presented. These pictures were paired with pictures of alcoholic beverages and non-alcohol beverage pictures (i.e. milk, orange juice). The IAPS pictures and the beverage pictures appeared simultaneously, side-by-side. This recently developed composite fMRI task allows independent assessment of responses to affective stimuli, alcohol associated cues, and the interaction of these two factors, as well as direct comparisons between conditions. Scrambled images were used as the control condition and were displayed during the inter-stimulus-interval (ISI). The scrambled images were derived from the IAPS images using a script that introduced a random phase shift into Fast Fourier Transformations (FFT) of each image, which preserved overall brightness and color but did not contain recognizable features. The presentation of the stimuli was jittered, so that the inter-stimulus interval (ISI) ranged from 0 to 15 sec. The pictures were presented in a random order in one run lasting 9 min and 30 sec. Stimuli were presented using a Linux laptop computer with in-house stimulus delivery software. They were projected using an Epson MP 7200 LCD projector onto a

screen placed at the foot of the MRI scanner bed and were viewed using a mirror mounted on the head coil. Each stimulus presentation lasted 800 msec. Participants were instructed to attend to the pictures.

fMRI acquisition: Imaging was performed using a 3 T General Electric MRI scanner (General Electric, Milwaukee, WI) and a 16 channel head coil. In-plane resolution was 3.75 x 3.75 mm. Functional scans were acquired using a $T2^*$-sensitive echoplanar sequence with a repetition time (TR) of 400 msec, echo time (TE) of 40 msec, and flip equal to 30°. We collected eight 5.0 mm contiguous axial slices drawn from the base of the orbitofrontal cortex upward to the level approximately at the top of the corpus callosum, which allowed us to image most of the temporal and ventral frontal lobe, as well as the ventral visual stream. A total of 1430 volumes were collected. Structural scans were acquired using a T1-weighted MP-RAGE (magnetization-prepared rapid gradient echo) sequence (TR, 100 msec; TE, 7 msec; flip, 90°), which facilitated localization and co-registration of functional data.

fMRI analysis: Analyses focused on changes in BOLD signal contrast (hereafter, activation) that occurred as the participants viewed the positive and negative pictures. Analyses were conducted using Analysis of Functional Neural Images (AFNI) software (Cox 1996). Echoplanar image volumes were preprocessed as follows: (1) voxel time series were interpolated to correct for non-simultaneous slice acquisition within each volume (using sinc interpolation and the most inferior slice as a reference), and (2) volumes were corrected for motion in three-dimensional space. Motion-correction estimates indicated that no participant's head moved >1.0 mm in any dimension from one volume acquisition to the next. We imposed a 6 mm full-width half-maximum (FWHM) smoothing kernel in the spatial domain. (3) We created a mask so that all of the background values outside of the brain were set to zero, so that we could calculate the percentage signal change in each voxel. This analysis was conducted in two stages. First, statistical maps were generated for each individual separately by linear contrasts of the regressors

of interest and the scrambled condition. Preprocessed time series data for each individual were then analyzed by multiple regression, which allowed co-variation of variables related to head motion and scanning run. The regression model consisted of the regressors of interest and six regressors of no interest modeling residual motion. Regressors of interest were convolved with a gamma-variate function that modeled a prototypical hemodynamic response before inclusion in the regression model (Cohen 1997). Idealized signal time courses were time-locked to image onset. Anatomical maps of *t* statistics representing each of these regressors of interest were spatially normalized by warping to Talairach space and combined into a group map. We applied a family-wise error rate correction (using a Monte Carlo simulation) to rule out false positives, yielding a corrected type 1 error < 0.05. Only activated volumes greater than 703 mm^3, or clusters larger than 10 voxels were considered significant. Second, we calculated a statistical map of the activation within each group (LY686017-treated and placebo) for each stimulus condition. Each condition was compared to the baseline scrambled image, which served as the interstimulus interval. We then performed voxel-wise *t*-tests of the event-related β-coefficients calculated from the general linear model to test for differences between the LY686017-treated group and the placebo group for each condition.

3.3. Results

Safety and tolerability

LY686017 was well tolerated. No serious adverse events were encountered. A frequency comparison across 26 categories of adverse events, uncorrected for multiplicity of tests to avoid type II errors, did not reveal a higher incidence in the active treatment group for any event category.

Spontaneous cravings (AUQ)

Controlling for pre-treatment baseline and sex, there was a highly significant decline of cravings over time ($F = 37.05$, $p < 0.0001$), and a significant effect of treatment ($F = 4.4$, $p = 0.04$) on this outcome. The change from baseline on the AUQ shown in **fig. 3.1**, and baseline values are indicated in the figure legend.

Clinicians Global Impressions (CGI ratings)

Controlling for pre-treatment baseline, sex and BMI, there was for CGI severity a significant effect of treatment ($F = 11.9$, $p = 0.001$). Change scores from placebo lead-in baseline on the Severity scale are shown in **fig. 3.2**, and the baseline scores are indicated in the figure legend. Very similar results were obtained on the Improvement scale of the CGI (main treatment effect: $F = 8.4$, $p = 0.006$).

BOLD fMRI responses to affective stimuli

In response to the negative images paired with neutral beverage cues, the placebo group demonstrated robust activations in the right inferior frontal gyrus / posterior insula and parahippocampal gyrus, middle temporal gyrus, and several other regions (see **table 3.2**). The LY686017 group showed activation in the superior temporal gyrus and the fusiform gyrus, but showed deactivation in the lingual gyrus, precuneus, posterior cingulate, and insula. In a direct comparison, the LY686017 group had significantly less activation than the placebo group, indicating less brain activation in response to fearful images, in the middle temporal gyrus,

cuneus, insula, middle occipital gyrus, and inferior frontal gyrus (**fig 3.3**). There was no area where the LY686017 group showed higher activation.

In response to the positive emotional stimuli paired with the neutral beverage cues, the LY686017 group demonstrated activation in the anterior cingulate, thalamus, and lingual gyrus. The placebo group only activated one cluster, in the precuneus, and in fact showed deactivation in several brain regions, including the anterior cingulate, and the superior and inferior frontal gyrus. When we directly compared the two groups, we found that the LY686017 group had significantly higher activation to the positive images in the thalamus, caudate, lingual gyrus, and several temporal areas (**fig 3.4**). The placebo group did not show higher activation in any region. Furthermore, in a direct linear contrast between the groups, our results indicated that the LY68017-treated group had significantly greater activation than the placebo-treated group to the positive images, while the placebo-treated group had greater activation than the LY686017-treated group to negative images (**fig 3.5**).

In contrast to the emotional images paired with neutral beverage cues, BOLD activation in responses to alcohol associated cues were not consistently affected by LY606817 (data not shown).

3.4. Discussion

Alcohol consumption in alcoholism occurs in cycles; often, uncontrolled consumption is interspersed with intervals of abstinence. Relapse, or the return to uncontrolled alcohol use following abstinence, is an unfortunate outcome for many alcohol-dependent patients. Although our understanding of the causes of relapse is incomplete, three main categories of relapse-triggering stimuli have been identified: priming doses of the drug itself, exposure to alcohol associated cues, and stress / negative affect (Brownell et al 1986; Le et al 1998), all of which may lead to increased craving for alcohol. Craving, or the obsessive drive to seek out and find the drug, can be either conscious or unconscious, and is often associated with negative affect, one of the most common antecedents of relapse (Brownell et al 1986; McKay et al 1999). In fact, urges to drink induced by alcohol associated cues are closely correlated with concomitant levels of self-reported anxiety (Monti et al 1993).

As expected, the anxious alcoholics in this study showed a robust increase in BOLD activation in several brain regions involved in the processing of fear-inducing visual stimuli. These results are consistent with numerous studies showing that visual processing is enhanced by emotion (Vuilleumier 2005). Alcoholics who received LY686017 had less activation to negative images paired with neutral beverage cues than the placebo group in several brain regions associated with emotional response to visual stimuli. In particular, the LY686017 group had less activation in the insula, a brain region where activation has been shown to correlate with subjective measures of craving (Bonson et al 2002; Brody et al 2002; Wang et al 1999), and which has recently been implicated in the maintenance of addictive behavior (Naqvi et al 2007). In addition, the LY686017-treated group showed greater brain activation to the positive IAPS images paired with neutral beverage cues than the placebo treated group. This may reflect an overall shift in the balance between positive and negative emotion as indicated by the improvement detected by the CGI scale. While our design of paired stimuli limits our interpretation of these results because the stimuli were not purely negative or positive, but were

paired with neutral beverage cues, a recent study has shown similar areas of activation in response to negative and positive IAPS images alone in alcoholics (Heinz et al 2007). In addition, Heinz's report suggests that the greater activation we observed to positive images in the striatum and thalamus of treated alcoholics may predict less drinking over the next six months, while brain response to alcohol cues were not predictive.

In contrast with the images paired with the neutral beverage cues, the brain response to the images paired with alcohol cues was not affected by LY686017. We were able to replicate the results of our previous study (see Chapter 2), in that all alcoholic participants demonstrated greater activation to the negative images with the neutral cues than the negative images with the alcohol cues. This occurred in both the LY686017-treated group and the placebo-treated group, suggesting that the drug affects brain response to pleasant and unpleasant images but not to alcohol cues. There are several reasons why this could be the case. First, LY686017 appears to target the brain's stress circuitry. In animals, SP is released in response to psychological stressors (Holmes et al 2003), and in humans, blockade of NK1 receptors with the antagonist GR205171 reduced symptoms of social anxiety (Furmark et al 2005). While the IAPS images have been consistently shown to effectively alter psychophysiological parameters such as heart rate, skin conductance, and brain activity (Lang et al 1990; Lang et al 1993), the effect of alcohol cues on these parameters has not been studied. It is unclear how alcohol cues would affect stress systems in the brain.

Another possible reason that we could not show an effect of LY686017 on the response to alcohol cues could be the limitation of the stimuli themselves. Our previous study suggested that the alcohol cues were anxiolytic, not anxiogenic, but this could vary between individuals. A recent study suggests that alcohol cues may provide inconsistent data in an inpatient alcoholic population because these patients actively avoid alcohol-related stimuli (Townshend and Duka 2007). The study suggests that avoidance of alcohol cues is related to the loss of control over drinking in these patients, and it is possible that this issue was more pronounced in our study

population of anxious alcoholics. Finally, we purposely chose high-arousal IAPS images in order to maximize the brain response to these images. Although there are no normed, validated arousal ratings for the alcohol images, it is likely that they are far less arousing than the IAPS images. It is also possible that LY686017 is not sensitive enough to affect the brain response to the cues.

This is the first study to use pharmacological fMRI to assess brain response to affective stimuli in alcoholism. There are a number of limitations in using fMRI to assess drug effects; most prominently, that the drug itself may affect respiration or blood flow, causing global changes in vasculature. For example, caffeine is a vasoconstrictor, and will therefore reduce neural flow regardless of the specific task employed (Mulderink et al 2002), and this disruption of the BOLD response could lead to misinterpretation of the data. There are two main strategies that researchers have used to counter this issue. One strategy is to identify drug-induced changes in blood flow in the absence of any specific paradigm. Second, and the more common approach to accurately interpret neuronal changes, is to include a control task that is distinct from the main task of interest. A control task that activates regions outside of those that would be activated in the main task of interest indicates that the change in activation is task-relevant, and not an unrelated hemodynamic disruption (Wise and Tracey 2006). In the current study, there was no difference in visual response to the scrambled control images between the LY686017-treated group and the placebo group. Furthermore, we observed a double dissociation between the negative images, where the LY686017-treated group had greater activation than the placebo group, and the positive images, where the placebo group had greater activation than the LY686017-treated group. This differential response indicates that the modulation of activity we observed is task-dependent and stimulus-dependent, and not a global hemodynamic shift caused by the drug.

This study provides preliminary evidence that emotional processing in alcoholism can be modulated by a pharmacological agent. Furthermore, this study provides consistent data across a range of measures suggesting that NK1 antagonism might be clinically effective in the treatment

of alcoholism. In addition to the pharmacological fMRI data which suggests beneficial effects of LY686017, patients treated with the drug reported less craving for alcohol (measured by the AUQ) and were rated less severe in clinical symptoms (measured by the Severity scale of the CGI). Since our study was conducted specifically in anxious inpatient alcoholics, larger trials will be required to assess its effect across a broader patient population. Future studies should examine whether our results can be replicated in non-anxious subjects, or in less severe, outpatient alcoholics. It is unknown whether pathological activation of the SP and NK1 systems underlie alcoholism, but if this is indeed a risk factor for alcohol dependence, then NK1 antagonism may be a useful treatment option.

Table 3.1. Baseline characteristics of subjects. Data are given as counts, or as means ± SEM. TLFB: Time Line Follow Back. Heavy drinking refers to 5 or more standard drinks in a day for males, and 4 or more for females. A standard drink is 10 – 12g alcohol. AUQ: Alcohol Urge Questionnaire. CPRS-SA: Comprehensive Psychopathological Ratings Scale, Self-Affective version (see Methods).

	Placebo (n = 25)	LY686017 (n = 25)
Males / females	16 / 9	21 / 4
Age	40.9 ± 2.0	42.0 ± 2.0
BMI	26.3 ± 1.1	26.2 ± 1.1
Spielberger Trait Anxiety	54.5 ± 2.5	54.0 ± 1.6
Alcohol Dependence Severity	24.1 ± 1.8	21.1 ± 1.6
TLFB: heavy drinking days in preceding 90 days period	65.6 ± 4.8	62.5 ± 2.1
TLFB: average drinks per drinking day	12.4 ± 1.2	12.8 ± 1.6
Baseline AUQ	19.1 ± 2.4	19.7 ± 2.3
CPRS-SA Depression	6.2 ± 0.8	6.0 ± 0.7
CPRS-SA Anxiety	11.9 ± 1.6	11.5 ± 1.3

Table 3.2. Brain Response of Patients on Drug and Placebo to Negative and Positive Images Without Alcohol Cues.

Condition	Group	Region	Volume (mm^3)	Talairach coordinates			*t*-score	*p*-value
				x	*y*	*z*		
Negative	LY686017	L Lingual gyrus	3560	-5	-45	-2	-3.418	< .005
		L Superior temporal gyrus	1016	-49	3	-14	3.656	< .005
		R Fusiform gyrus	952	41	-55	-6	3.124	< .005
		L Insula	744	-40	-29	24	-3.463	< .005
	Placebo	L Parahippocampal gyrus	8448	-35	-19	-8	3.309	< .005
		R Middle temporal gyrus	4480	53	-41	0	3.939	< .001
		R Inferior frontal gyrus	4168	49	25	-6	3.892	< .005
		L Caudate	2000	-31	-39	6	-3.412	< .005
		R Superior frontal gyrus	1736	19	57	0	3.155	< .005
	LY686017 > Placebo	No clusters detected						
	Placebo > LY686017	L Middle temporal gyrus	1688	-57	-41	-6	3.375	< .005
		R Cuneus	1064	7	-75	32	2.995	< .005
		L Insula	928	-45	-31	22	3.244	< .005
		L Middle occipital gyrus	808	-53	-77	2	2.976	< .005
		R Inferior frontal gyrus	776	45	37	-16	3.187	< .005
Positive	LY686017	R Anterior Cingulate	2888	1	-1	0	4.284	< .001
		R Thalamus	1144	1	-25	2	3.35	< .005
	Placebo	L Anterior Cingulate	8432	-23	35	6	-4.076	< .001
		L Precuneus	1232	-27	-61	30	3.194	< .005
		R Medial frontal gyrus	1168	3	55	-14	-3.738	< .005
		L Inferior temporal gyrus	1056	-61	-47	-14	-3.396	< .005
	LY686017 > Placebo	R Caudate	1864	1	-1	2	3.07	< .005
		L Middle temporal gyrus	712	-57	-17	-4	3.097	< .005
	Placebo > LY686017	No clusters detected						

Threshold is set at $p < 0.005$ uncorrected and a voxel threshold of at least 10 active voxels which yields a family-wise error rate correction of $p < .05$.

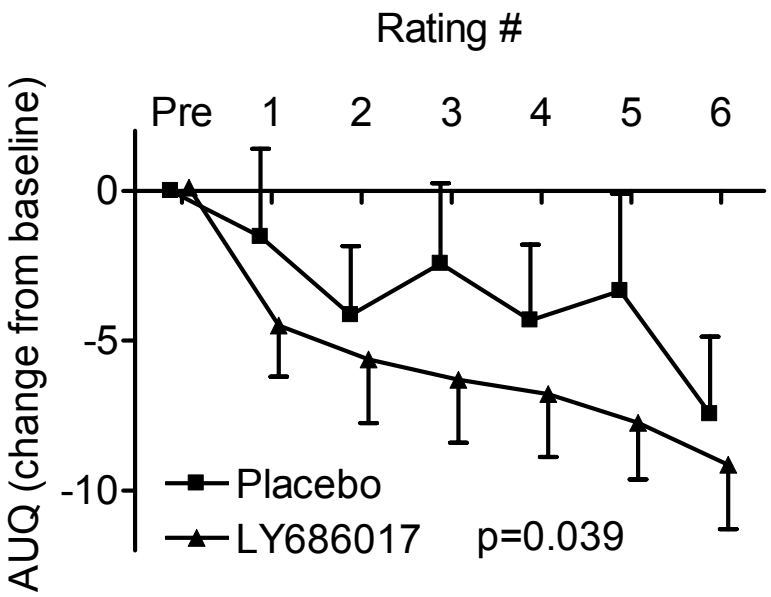


Fig. 3.1. Change from baseline for spontaneous alcohol cravings, as measured by scores on twice weekly ratings with the Alcohol Urge Questionnaire (AUQ). The first value reflects the baseline rating obtained during the placebo lead-in week. The following ratings are from the three week phase during which patients received placebo or active drug (Ratings 1-2: first; 3-4: second; and 5-6: third treatment week. Data are means±SEM. Baseline values for placebo and LY686017 groups were 19.1±2.4 and 19.7±2.3, respectively. Minimum rating on the scale is 8.

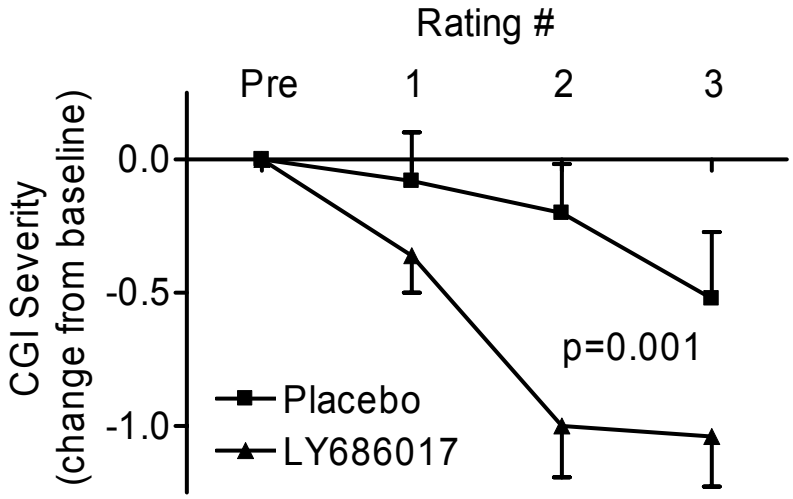
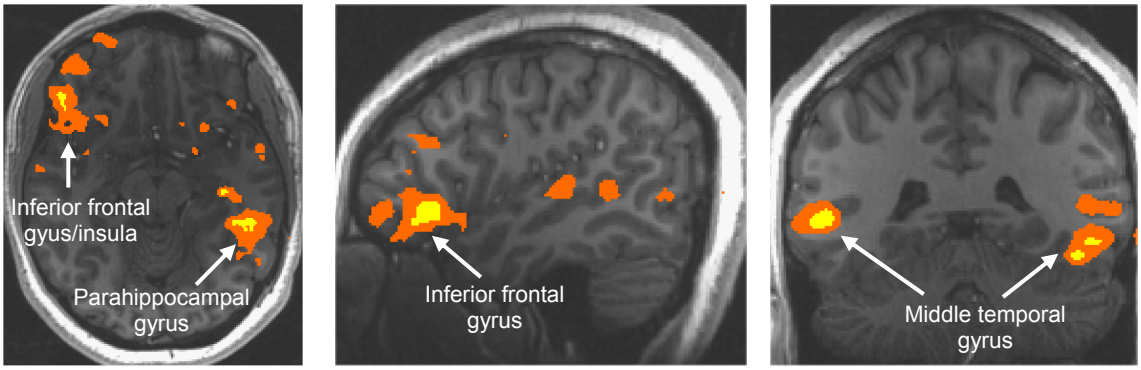


Fig. 3.2. Change from baseline on weekly observed based ratings using the Severity scale of the Clinicians Global Impression (CGI) rating questionnaire. The first value reflects the baseline rating obtained during the placebo lead-in week that preceeded the three week long phase during which patients received placebo or active drug. Average baseline ratings for the placebo and LY686017 groups were 3.80 ± 0.15 and 3.88 ± 0.13, respectively. Data are means ± SEM. Similar results were obtained for the Improvement subscale.

A)



B)

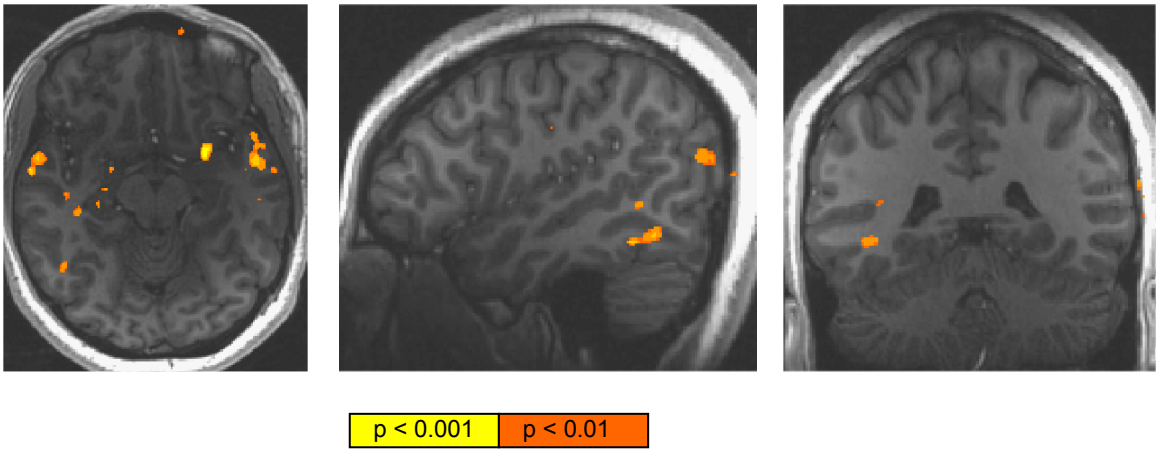


Fig 3.3. BOLD responses to visual negative affective stimuli. A) In the placebo group, there were robust activations in the middle temporal gyrus, cuneus, insula, middle occipital gyrus, and inferior frontal gyrus. B) The LY686017-treated group had significantly less activation in these areas, indicating less brain activation in response to fearful images. Group statistical maps are superimposed upon a T1 structural image in Talairach space. See **table 3.2** for values.

A)

B)

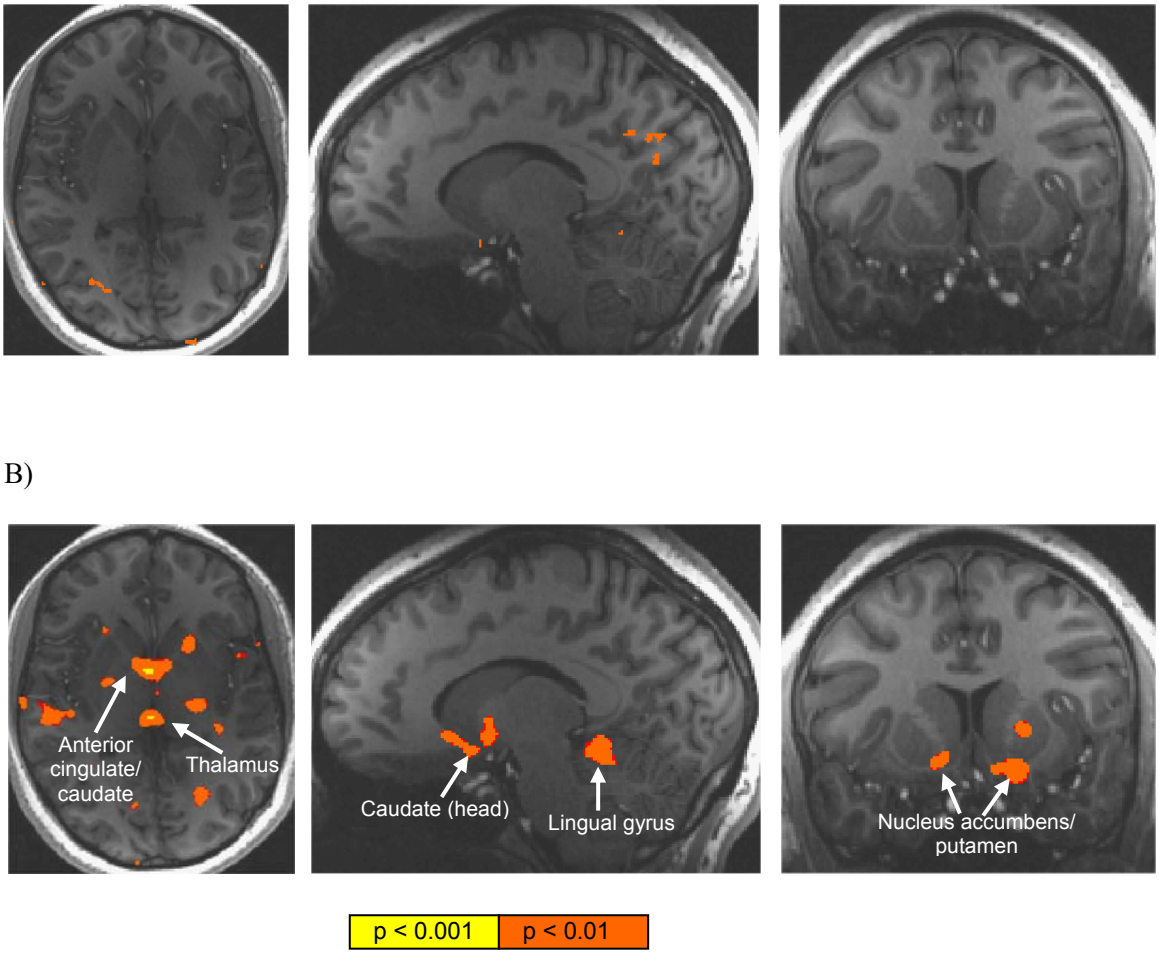


Fig 3.4. BOLD responses to visual positive affective stimuli. A) The placebo group had very little activation in response to the positive emotional stimuli. B) In contrast, the LY686017-treated group had robust activation in the anterior cingulate, thalamus, caudate (including ventral putamen), lingual gyrus, and several temporal areas. Group statistical maps are superimposed upon a T1 structural image in Talairach space. See **table 3.2** for values.

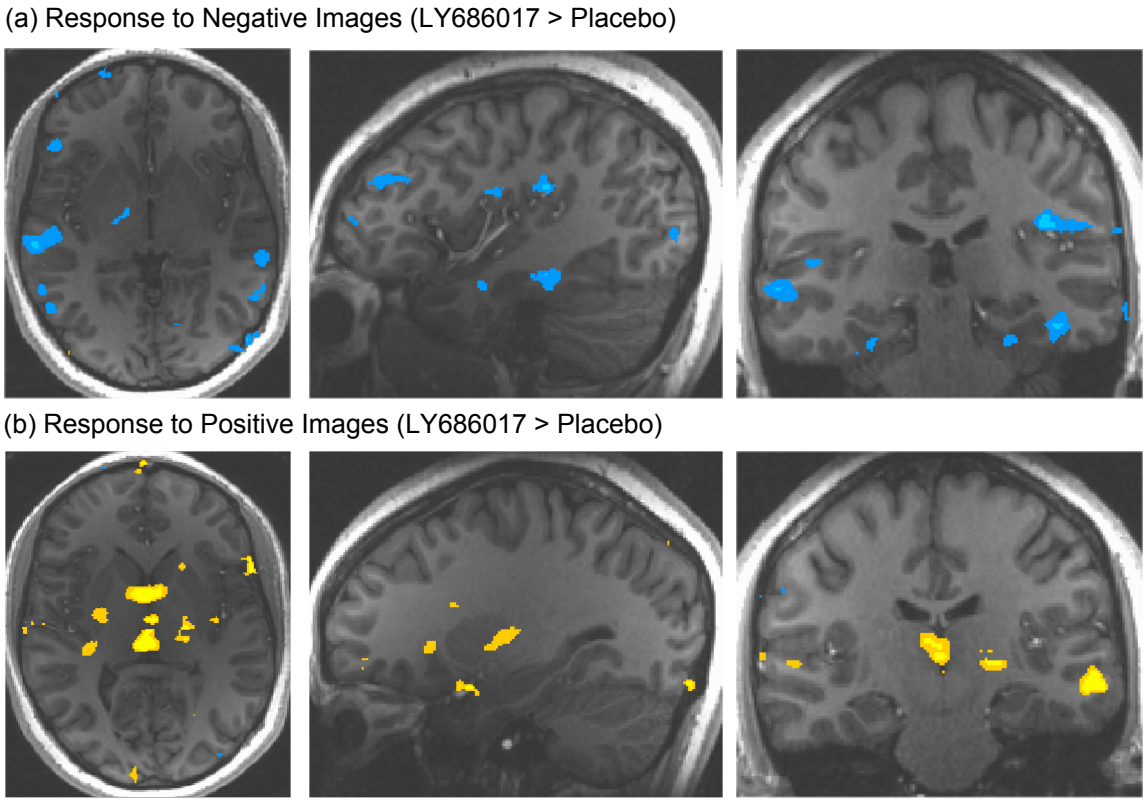


Fig. 3.5. Direct comparisons in BOLD response between the drug and placebo groups to negative (a) and positive (b) emotional images. Blue indicates stronger activation in the placebo group, while yellow indicates stronger activation in the LY686017-treated group ($p < 0.05$). The LY686017-treated group had significantly less activation to the negative images than the placebo group, indicating less brain activation in response to fearful images, in the middle temporal gyrus, cuneus, insula, middle occipital gyrus, and inferior frontal gyrus. In response to the positive emotional stimuli, the LY686017-treated group had significantly higher activation than the placebo group in the caudate, and the left middle temporal gyrus. Group statistical maps are superimposed upon a T1 structural image in Talairach space. See **table 3.2** for values.

Chapter 4:

Modulation of Brain Response to Emotional Images by a Cognitive Decision-Making Task

We have demonstrated that alcoholic patients have a greater response to negative images and a reduced response to positive images relative to control participants, and we have demonstrated that this difference can be modulated by alcohol cues and by treatment with LY686017. Both of the previous experiments employed passive-viewing paradigms, in which participants were not required to cognitively engage in the task. Previous neuroimaging studies indicate that task requirements may alter neural activation, and we hypothesize that requiring alcoholic patients to make cognitive or emotional judgments about emotional images will intensify differences in activation from controls. We expect to find the largest differences in the frontal lobe, which is a brain region involved in decision-making, as well as an area of the brain that is particularly vulnerable to alcohol's neurotoxic effects.

4.1. Introduction

We have previously reported that alcoholic patients exhibit increased neural responses to negative images in brain areas implicated in visual processing of emotional images, such as the lingual gyrus, thalamus, and parahippocampal gyrus. These results are consistent with studies that have shown increased attention to negative stimuli among patients suffering from depression, phobias, anxiety disorders, and panic disorders (Bradley et al 1997; Lang and Sarmiento 2004; Mathews et al 1996; Spector et al 2003). We also reported that alcoholics exhibited a decreased response to positive images relative to controls. Our previous experiments employed simple passive-viewing tasks, and did not elicit robust frontal lobe activation from either alcoholics or healthy controls. We designed the current experiment to parametrically vary the demands of the task in order to better engage frontal brain systems.

Impaired emotional regulation in alcoholics may be related to brain atrophy. Although prolonged alcoholism causes diffuse brain damage, the frontal lobe is particularly susceptible to alcohol-induced atrophy (Moselhy et al 2001; Oscar-Berman and Marinkovic 2003; Pfefferbaum et al 1997). Many of the brain regions vulnerable to the neurotoxicity of alcohol have also been implicated in emotional processing. The orbital and medial prefrontal cortices in particular have been implicated in the production of emotional states (Phillips et al 2003). These regions activate in response to the perception of pleasant and unpleasant odors (Francis et al 1999), flavors (O'Doherty et al 2001) and tactile stimuli (Zald et al 1998), as well as during the evaluation of moral dilemmas (Greene et al 2001), which indicate that these areas may be involved in the autonomic regulation of emotional states (Phillips et al 2003). The ventrolateral prefrontal cortex has been shown to activate in response to the induction of sad mood and guilt (Pardo et al 1993; Shin et al 2000), the recall of emotional memories (Reiman et al 1997), and in response to facial expressions of negative emotion (Sprengelmeyer et al 1996).

In the current study, we hypothesized that alcoholics will show differential activation relative to controls in frontal lobe regions when engaged in a cognitive or emotional decision-making task. Previous research has shown that when participants cognitively label the emotional expression of fearful faces, amygdala activation is reduced, while the activation in the right prefrontal cortex, a brain region implicated in the regulation of emotional responses, is increased (Hariri et al 2000). A study using aversive images also found decreased amygdala and increased prefrontal activity when participants were asked to rate the images (Taylor et al 2003). Other studies, however, have found an increase in limbic activation during a rating condition relative to a recognition condition (Keightley et al 2003; Liberzon et al 2000). One possible reason for the inconsistent reports in the literature is that many studies have used a control condition that has actively inhibited the emotional response. In these studies, participants have been asked to attend to the location of the scene, which is a task intended to control for attentional demands across conditions (Gusnard et al 2001; Lane et al 1997a; Ochsner et al 2004), but may inadvertently suppress emotion by requiring the participant to attend to a characteristic of the image that is not related to its emotional content.

In the current experiment, three levels of emotional processing were investigated. In one condition, the participants simply pressed a button when they saw an emotional image. In a second condition, the participants were instructed to make a non-emotional, cognitive decision about the location of the image. In the third condition, the participants were instructed to make an emotional judgment about the picture, involving their own affective response to the image. Using this design, we investigated the role of an attention-demanding task (judging the location of the image) separately from the role of an emotionally demanding task (judging subjective feelings about the image). If the frontal lobes are involved in the emotional evaluation of the images, we expected to see differential activation in frontal brain systems between alcoholics and controls during the decision-making tasks, which may suggest that the frontal lobe impairments

often observed in alcohol-dependent individuals may relate to emotional deficits observed in alcoholics.

4.2. Methods

Participants

Fifteen alcohol-dependent patients and fifteen healthy controls participated in this study (see **table 4.1** for demographic information). Alcohol-dependent patients were recruited from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) inpatient unit at the Clinical Center of the National Institutes of Health in Bethesda, MD. All participants were interviewed using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (First 1996). Information on recent and chronic alcohol use was obtained from structured research questionnaires. All alcoholic patients met DSM-IV criteria for alcohol dependence. We excluded patients who met the criteria for alcohol abuse but not alcohol dependence, as well as those who had a history of delirium tremens or gross neurological disorders. In addition, we excluded patients who had an IQ less than 80 or who demonstrated signs of dementia or Korsakoff's disease. Participants were not thiamine deficient at admission, and none of the subjects had a history of head injury requiring hospitalization. Patients were scanned three weeks after admission. Healthy community-recruited male participants with no history of significant medical illness or psychiatric disorders were included for comparison. All subjects were assessed with the Structured Clinical Interview for DSM-IV, which confirmed that each alcoholic patient met criteria for alcohol dependence and that no control participant met criteria for a psychiatric disorder. Healthy controls were compensated monetarily. All participants were right-handed and had normal or corrected-to-normal vision. All subjects provided written informed consent to participate in the study which was approved by the NIAAA Institutional Review Board.

Visual stimulation and task

Visual slides were chosen from the International Affective Picture System (IAPS) (Lang, Bradley & Cuthbert, 1993) which has been used extensively in both healthy and clinical samples. Normed ratings of valence and arousal exist for each of the slides in the IAPS, which allowed for precise selection of matching slide sets. Forty high-arousal negative pictures and 40 high-arousal positive pictures were presented. The average valence for the negative images was 2.69 (SD = 0.74), and the average valence for the positive images was 6.58 (SD = 0.85). We purposely chose non-ambiguous images that were clearly negative (i.e. burn victims, attack dogs, violent scenes) or positive (i.e. babies, flowers, nudes) to ensure that the task was simple for the participants. We chose highly arousing images (negative arousal = 6.01, SD = 0.76, positive arousal = 5.49, SD = 0.86) in order to elicit maximal brain response. Negative and positive images were not significantly different in arousal rating.

Scrambled images were used as the control condition and served as the inter-stimulus-interval (ISI). The scrambled images were derived from the IAPS images using a script that introduced a random phase shift into Fast Fourier Transform (FFT) of each image. This resulted in scrambled pictures having the same power spectrum as the original ones but no visually recognizable similarities. The ISI ranged from was 0 msec – 8 sec.

The experiment was presented in 3 blocks. The order of the blocks was counterbalanced. In the emotion block (EB), participants were instructed to press one button if they "liked" the image or found it to be pleasant, and a second button if they "disliked" the image or found it to be unpleasant. In the cognitive block (CB), they were asked to press one button if the scene took place indoors, and a second button if the scene was located outdoors. In the passive block (PB), the participants were not required to make a judgment, but were asked to press a button when they saw a picture, to control for attention and motor effects. The button box was attached to a computer that allowed us to measure reaction time for each individual trial.

Stimuli were presented using a Linux laptop computer with in-house stimulus delivery software, and were projected inside the fMRI scanner using an Epson MP 7200 LCD projector for subject viewing. Each stimulus presentation lasted 2.0 sec (one volume acquisition).

Subjective ratings were collected at baseline (prescan) and immediately following each scan (postscan) for each subject. Participants were asked to subjectively rate their subjective experience of mood on a five point scale using the Positive and Negative Affect Scales (Watson et al 1988).

fMRI acquisition.

Imaging was performed using a 3 T General Electric MRI scanner (General Electric, Milwaukee, WI) and a 16-channel head coil. We collected 16 contiguous 5.0 mm thick axial slices with a 1 mm gap drawn from the base of the orbitofrontal cortex upward to the top of the brain (in-plane resolution 3.75 x 3.75 mm), providing whole-brain coverage including subcortical regions of interest. Whole-brain high-resolution coronal structural scans were collected using a T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) pulse sequence, which facilitated localization and co-registration of functional data (matrix 256 x 256 x 124, repetition time (TR) = 100 ms, echo time (TE) = 12 ms, field of view (FOV) = 24 cm). Functional scans were acquired using a $T2^{*}$-EPIRT sequence that measure changes in blood oxygen level dependent (BOLD) contrast (approximately 260 volumes, TR = 2s, echo time (TE) = 40 msec, flip angle = 30°, matrix 64 x 64, in-plane matrix = 128, FOV = 24 cm, slice thickness = 5 mm). To allow for signal stabilization before events of each run, 10 acquisitions were obtained before task onset.

fMRI analysis.

Analyses were conducted using Analysis of Functional Neural Images (AFNI) software (Cox 1996). Echoplanar image volumes were preprocessed as follows: (1) voxel time series were interpolated to correct for non-simultaneous slice acquisition within each volume (using sinc interpolation and the most inferior slice as a reference). (2) Volumes were corrected for motion in

three-dimensional space. Motion-correction estimates indicated that no participant's head moved >1.0 mm in any dimension from one volume acquisition to the next. We imposed a 6 mm full-width half-maximum (FWHM) smoothing kernel in the spatial domain. (3) A mask was created so that all of the background values outside of the brain were set to zero. This allowed the calculation of the percentage signal change in each voxel. Preprocessed time series data for each individual were then analyzed by multiple regression, which allowed co-variation of variables related to head motion. The regression model consisted of the orthogonal regressors of interest and six regressors of no interest modeling residual motion after volume registration. Regressors of interest were convolved with a gamma-variate function that modeled a prototypical hemodynamic response before inclusion in the regression model (Cohen 1997). Idealized signal time courses were time-locked to image onset. Statistical maps were generated for each individual separately that consisted of an event-related β-coefficient and a t-statistic representing each of the regressors of interest in each run. Regressors of interest were the negative and positive images of each block. Because of the short duration of each run, we did not do any band-pass filtering or de-trending of the data. Each of the three runs was analyzed separately.

Anatomical maps of *t* statistics and β-coefficient were spatially normalized by warping to Talairach space. We conducted two separate analyses. In the first analysis, the negative and positive images were combined (in order to increase statistical power and to investigate processing of emotion across valence categories). A statistical map was computed using the general linear model (GLM) in a three-factor voxel-wise mixed-model ANOVA, where the fixed factors were group (alcoholic or control) and task type (passive, emotional, or cognitive judgment), and subject was a random factor. The second analysis examined the response to negative and positive images separately (in order to identify how BOLD response was affected by valence). In this analysis, we performed voxel-wise *t*-tests between alcoholics and controls, using the event-related β-coefficients for each of the conditions separately for the negative and positive images.

We corrected for multiple comparisons by using a family-wise error rate correction (Monte Carlo simulation) to rule out false positives, which resulted in a corrected type I error < 0.05. When reporting ANOVA and t-test results, we only report clusters larger than 8 voxels at an individual voxel threshold of $p < 0.001$.

Where we observed significant differences between groups and across conditions, we characterized the actual BOLD signal change in a volume-of-interest (VOI) analysis in which time series signal data from the same brain coordinates in both groups were analyzed. These VOIs were chosen *post-hoc*, based on brain regions that differed significantly between the two groups, in order to investigate the source of effects that were driving these differences. The VOIs were drawn as spheres with a radius of 5 mm. Within each region, we generated a mean impulse response function (IRF) for each of the stimulus types in each run. The IRF was extracted from the time series as follows: (1) signal at each voxel was converted to a (percentage) deviation from the mean for that voxel across the entire time series, (2) signal was averaged by stimulus type and spatially translated into Talairach space, and (3) IRFs were estimated by generating a 5 TR (or 10 second) time course following the presentation of each stimulus category, which estimated 5 coefficients, covering lags from 0 to 10 sec after stimulus presentation. Next, a mask was created consisting of the volume of interest through which each individual participant's data was extracted.

These data were subject to analysis of variance using the general linear model (package JMP-SAS; SAS Institute, Cary, North Carolina). We entered the peak percent signal change of the hemodynamic curve of each subject in each region into our model as the dependent variable. Independent variables were group (alcoholic or control), emotion (positive or negative), and condition (passive, cognitive, or emotional), and all possible two- and three-way interactions. Where we observed significant main effects or interactions, we conducted student's t-tests to clarify differences. The *p*-value for significance (two-tailed) was set at 0.05.

We also ran correlational analyses (package JMP-SAS) in order to investigation whether the peak signal change in the VOIs was correlated with either reaction time or number of errors in the cognitive and emotional decision-making blocks.

4.3. Results

Behavioral Results

Alcoholics did not significantly differ from controls in the number of errors committed in either the CB ($F = 0.993$, $p = 0.33$) or the EB ($F = 2.32$, $p = 0.141$), or during the entire experiment ($F = 2.82$, $p = 0.107$). See **fig 4.1** for values. Differences in reaction time between alcoholics and controls reached trend-level significance in the CB ($F = 4.249$, $p = 0.049$) and the EB ($F = 3.895$, $p = 0.0592$), but there was no difference in reaction time to the PB (**fig 4.2**).

Alcoholics reported higher levels than controls of both negative affect ($F = 8.57$, $p = 0.0069$) and positive affect ($F = 12.89$, $p = 0.0013$), measured by the PANAS.

Brain activations by linear contrasts between conditions

Significant within-group differences in activation among conditions are listed in **table 4.2**. In the CB compared to the PB, controls showed increased activation in the left postcentral gyrus and the left parahippocampal gyrus. Alcoholics demonstrated increased activation to the CB in several frontal regions, including the right medial frontal gyrus, right middle frontal gyrus (MFG), left inferior frontal gyrus (IFG), and left superior frontal gyrus (SFG). Alcoholics also showed increased activation in the left postcentral gyrus and the bilateral precuneus (**fig 4.3**). Results of voxelwise *t*-tests comparing the activation in alcoholics with that of controls are listed in **table 4.3**. *T*-tests indicated that alcoholics showed more activation than controls in the left IFG, right postcentral gyrus, left putamen, left SFG, and left MFG. There were no regions where controls showed more activation than alcoholics during the cognitive task (**fig 4.4**).

In the EB, controls showed increased activation relative to the PB in the left precuneus, left parahippocampal gyrus, and right middle occipital gyrus. As in the CB, the EB compared to

the passive viewing yielded significant increased activation in the alcoholics in several regions, including the insula, left posterior cingulate, left postcentral gyrus, right MFG, left fusiform, and right middle occipital gyrus (MOG) (**fig 4.5**). The controls demonstrated more activation in the EB than the PB in the left precuneus, left parahippocampal gyrus, and right MOG. Voxelwise *t*-tests indicated that the alcoholics had greater activation than the controls during the EB in the left inferior frontal gyrus, bilateral putamen, left SFG, right medial frontal gyrus, and left superior frontal gyrus. There were no clusters detected where controls had greater activation than alcoholics (**fig 4.4**).

When we compared the EB to the CB in controls, no significant differences were found. The alcoholic patients demonstrated greater activation to the EB than the CB in the left medial frontal gyrus (coordinates -6, 49, 19; *t*-score = 7.59).

Brain activations by linear contrasts of each emotional valence

In this analysis, we examined compared activation in the controls with those in alcoholics for each emotion type separately. Significant differences are listed in **table 4.4**. In the PB, controls had greater activation than alcoholics to the positive images in the right precuneus and left parahippocampal gyrus, while alcoholics had greater activation than controls to the negative images in the bilateral middle temporal gyri, and in the right superior and inferior frontal gyri. In the CB, alcoholics had greater activation than controls irrespective of the emotional valence in several frontal lobe regions, including the bilateral middle frontal gyri and left IFG. Alcoholics also had greater activation than controls to the negative images in the bilateral putamen. Controls demonstrated greater activation than alcoholics in only one region, the left inferior parietal lobule, during the presentation of positive images. In the EB, the alcoholics had greater activation than the controls to both the negative and positive images in several frontal, temporal, limbic, and motor regions, including the pre- and post-central gyri, thalamus, putamen, insula, and posterior cingulate.

Signal change in selected post-hoc *volumes of interest*

In an exploratory analysis, we further examined regions that demonstrated significant differences between alcoholics and controls by extracting data through volumes-of-interest, drawn *post hoc*, to further explore task-dependent activation. Those regions included the left IFG (Talairach coordinates -46, 13, 20), the left STG (-50, -51, 17), the right middle frontal gyrus (38, 2, 55), and the left insula (-31, -30, 12). In each of these regions, alcoholics exhibited robust increased activation during the emotional and cognitive tasks compared to the passive task, while control participants did not exhibit significant differences among task conditions. Graphs of the percent signal change relative to baseline for controls and alcoholics in each condition in each of the four selected VOIs are shown in **fig 4.6**.

Left inferior frontal gyrus. In the IFG, alcoholics showed higher activation than controls across conditions ($F = 40.76$, $p < 0.0001$). We also found a significant difference across conditions ($F = 4.84$, $p = 0.013$), and subsequent students *t*-tests indication that subjects had more activation to the CB than the PB ($p = 0.0035$), and higher activation to the EB than the PB ($p = 0.0075$). There was no main effect of emotion, and no significant interactions, although the group x condition interaction reached trend-level significance ($F = 2.778$, $p = 0.065$).

Left superior temporal gyrus. In the STG, alcoholics had significantly greater activation than controls ($F = 11.63$, $p = 0.0008$). There was also a main effect of condition ($F = 5.712$, $p = 0.004$), and student's *t*-tests showed that activation to the CB was significantly greater than to the PB ($p = 0.0006$), and activation to the EB was greater than to the PB ($p = 0.014$). There was also a significant group x condition interaction ($F = 4.831$, $p = 0.009$), and post-hoc student's *t*-test revealed that alcoholics had higher activation in the EB and the CB than they did in the PB, and that alcoholics had higher activation in the EB and CB than controls in any condition ($p > 0.05$). There were no main effects or interactions with emotion.

Right middle frontal gyrus. In the MFG, alcoholic patients demonstrated greater activation than controls across conditions and emotional valence ($F = 63.428$, $p < 0.0001$). There was also a

main effect of condition ($F = 4.484$, $p = 0.013$), and a group x condition interaction ($F = 4.524$, $p = 0.012$). Student's *t*-tests indicated that there was more activation to the CB than to the PB ($p = 0.0035$) and more activation to the EB than the PB ($p = 0.0075$). Subsequent *t*-tests of the group x condition interaction indicated that the alcoholics had higher activation in the EB and the CB than they did in the PB, and that alcoholics had higher activation in the EB and CB than controls in any condition ($p > 0.05$). There were no main effects or interactions with emotion.

Left insula. In the left insula, there was a main effect of group ($F = 5.366$, $p = 0.0218$), but no main effect of condition or emotion, and no interactions.

Behavioral correlations in selected post-hoc *volumes of interest*

In order to investigate whether brain activity was correlated with task difficulty, we ran correlations between peak percent signal changes in the VOIs and reaction time/error rates. After controlling for group (alcohol or control) and task type (CB or EB), reaction time and number of errors did not correlate with percent signal change in the IFG. We also did not find significant correlations in the STG. Longer reactions times did significantly predict activation in the right MFG after controlling for group and task type ($F = 5.73$, $p = 0.020$). We also found a significant positive association between activation in the MFG and error rate ($F = 10.96$, $p = 0.0017$). Insula activity did not correlate with reaction time, but it did significantly predict error rate ($F = 11.696$, $p = 0.0012$), demonstrating that higher errors rates were correlated with increased insula activation.

4. 4. Discussion

This study was designed to explore differences in the neural correlates of emotional processing between alcoholic patients and controls. During the passive viewing of high-arousal emotional images, alcoholics and controls did not differ in brain activation in frontal/limbic areas. However, during evaluation of emotional images, alcoholics exhibited greater activation than controls in frontal, striatal, and temporal regions. These differences in brain activation were observed in the absence of a difference in error rates. We suggest that the cognitive and emotional evaluation of emotional images and the requirement to generate a quick response may have been a more difficult for the alcoholics than the controls, as reflected in longer reaction times of the alcoholic patients. This task may have required alcoholics to recruit additional brain areas that controls did not need to complete the task accurately.

Several studies have demonstrated that neural response to emotion can be modulated by cognitive and attentional demands. Hariri and colleagues found that cognitive processing of faces reduced amygdala activation in healthy controls (Hariri et al 2000). Similarly, a PET study in which participants either passively viewed or emotionally rated IAPS images demonstrated an association between activity in the insula and amygdala and passive viewing, while cingulate cortex and the dorsal medial prefrontal cortex activation was associated with rating (Taylor et al 2003). In the current study, control participants did not demonstrate differential activation in the judgment conditions compared to the passive viewing condition in the amygdala or frontal areas. Our task, however, was less difficult than the task employed by Taylor and colleagues. In their study, subjects had to rate each image on a 10-point scale, from -5 to +5, whereas in our study, subjects only had to decide if they "liked" or "disliked" the image. The images chosen in the current experiment were at the extremes of the standardized ratings of negative and positive valence, so that there were no ambiguous pictures. It is possible that the controls in our study

were making an automatic, rather than an evaluative, decision, whereas the task may have required more effort from the alcoholics.

Differences in limbic regions

In contrast to controls, alcoholic participants demonstrated increased activation in several frontal and limbic structures in the judgment conditions relative to passive viewing. The insula was particularly strongly activated in the EB in the alcoholics, and this region has been implicated in the generation of affective states in response to aversive, especially disgust-related, stimuli (Phillips et al 1998). The insula reliably activates in healthy subjects during induced sadness and anticipatory anxiety, as well as during exposure to trauma-related stimuli in subjects with post-traumatic stress disorder (Charney 2002), and it is possible that the insula is activated more robustly during emotional decision-making in alcoholics than in controls. The cingulate, another area that was more highly activated in the EB relative to the PB in alcoholics but not in controls, is also implicated in the processing of negative emotion, particularly threat-related words (Maddock et al 2003). The greater activation of these regions in the alcoholics than the controls during emotional decision-making, especially during the presentation of negative images, could in part explain alcoholics' greater tendency to experience negative affect (Elkins et al 2006). In addition, the correlation observed between increased insula activation and increased error rates suggests that hyper-responsiveness of the insula may interfere with cognitive or emotional appraisal.

Differences in frontal regions

Studies have shown that introspection of emotional experience recruits frontal brain regions. For example, Lange and colleagues presented fearful and neutral facial stimuli to participants, who either passively viewed the faces, or made a judgment about either the gender or the emotional expression of the face (Lange et al 2003). They found activation in the left ventral frontal cortex in the gender and emotion judgment conditions, but not in the passive

condition. In the current study, we found that the alcoholics had greater activation than controls in a number of frontal brain regions in the CB and EB.

The greater activation observed in the alcoholics is largely left-lateralized, which suggests that the activation could be related to language, or more specifically, to internal speech. As a task becomes more difficult, both children and young adults are more likely to use internal speech, the activity of talking to oneself, to complete the task (Beaudichon 1973; Duncan 1997; Duncan 2002). The left inferior frontal gyrus (IFG) is involved in private speech use (see (Morin and Michaud 2007) for review), and becomes active when participants are instructed to silently articulate sentences or words (McGuire et al 1996a; McGuire et al 1996b). Emotion tasks that require participants to evaluate their emotional response to a stimulus (Gusnard et al 2001; Lane et al 1997a; Ochsner et al 2004), as well as tasks which require non-emotional self-referential judgments (Johnson et al 2005; Paulus and Frank 2003; Seger et al 2004) also reliably activate the IFG, likely because these tasks often engage participants in private speech. Alcoholics also had greater activation than controls in the left superior frontal gyrus, another area that is activated during language tasks when participants are listening to speech (Price 2000).

Alcoholic patients also demonstrated greater activation than controls in the right middle frontal gyrus. This area may be more involved in attention than emotion (Yamasaki et al 2002), which may explain why the right MFG was activated in both the cognitive and emotional task. The activity of the right middle frontal gyrus correlated positively with both reaction time and error rates, suggesting that activity in this region may be related to impaired appraisal of emotional images. The left superior frontal gyrus, a region that has been implicated in the monitoring and manipulation of short-term working memory (du Boisgueheneuc et al 2006), as well as in speech perception and production (Saykin et al 1999), was also more activated in alcoholics than controls. This region is also close to the frontal eye fields, and therefore activation in this region in the alcoholics could be related to greater preparation to make scanning eye movements.

Previous neuroimaging studies suggest that greater effort may be reflected in increased neural activation. For example, in a study of bilingual English and Mandarin-speaking participants, researchers found greater activation in the left prefrontal and parietal regions when the participants read words in their less proficient language, and suggested a link between lesser proficiency, longer reaction times, and greater BOLD signal change in these areas (Chee et al 2001). Other studies have shown that BOLD activity in the prefrontal cortex parametrically varies with increased working memory load (Rypma et al 1999; Smith and Jonides 1997). In alcoholism, previous fMRI studies have reported differences in activation patterns between alcoholic patients and controls in the absence of performance differences. Alcoholic patients exhibit greater activation in the cerebellum during working memory tasks, even though they perform similarly to controls, which suggests that alcoholics require greater activation than controls to maintain the same level of performance (Desmond et al 2003). Pfefferbaum et al (2001) also found that alcoholics exhibited different patterns of brain activation than controls during a working memory task despite equal performance, and speculated that chronic alcohol exposure may lead to a reorganization of brain circuitry in order to compensate for deficits caused by alcoholism (Pfefferbaum et al 2001).

Conclusions

This is the first experiment to examine the neural correlates of emotional decision-making in alcoholic patients. Our experimental design allowed us to parametrically vary the difficulty of the task and control for attentional demands. At the lowest level of difficulty (the PB), there were very few differences between alcoholics and controls. As the difficulty of the task increased, however, differences in activation emerged in several brain regions, but particularly the frontal lobe. This occurred both when the participants were asked to attend to their emotions (in the EB), and when they were asked to attend to the location of the image (in the CB). The increased reaction time in both the alcoholics and controls suggests that the task difficulty did increase from the PB to the CB and EB. Since performance on the task was

matched for alcoholics and controls, differences in task performance cannot explain the differences observed in neural activation. As the same level of performance, it is likely that the alcoholics recruited additional brain regions in order to complete the task quickly and accurately.

Similar regions were recruited in the cognitive and emotional blocks for negatively and positively valenced images, which suggests that emotional dysfunction is not specific to one emotion type. In the PB, however, the controls demonstrated greater activation to the positive images, whereas the alcoholics demonstrated greater activation to the negative images. This is consistent with previous studies we have run examining brain response of alcoholics to IAPS images. The greater activation of the alcoholics to both negative and positive images in the decision-making tasks may be more related to task demands than to emotional valence.

A limitation of this study is the absence of non-emotional control images. We chose to study high-arousal negative and positively valenced images in order to maximize brain activity in emotional circuits, but future studies should examine whether the frontal lobe is recruited when alcoholics make decisions about non-emotional images as well. Another limitation of this study is the absence of an empirical measure of frontal lobe atrophy in the alcoholic patients. Although it is well-known that prolonged alcohol consumption causes atrophy of frontal regions (Moselhy et al 2001; Oscar-Berman and Marinkovic 2003; Pfefferbaum et al 1997), and it is highly probable that the alcoholic patients in the current study suffer from atrophy, a measure of brain shrinkage in each patient would allow us to correlate frontal lobe activity with structural deficits.

This study demonstrates greater recruitment of frontal lobes during an emotional processing task in a clinical population. The requirement of frontal lobe engagement in the alcoholics even in a simple two-choice task suggests inefficiency in alcoholics' cognitive functioning and may offer insight into a possible reason for the impaired ability of alcoholics to regulate emotion at a more complex level. More complicated emotional decision-making may tax already weak frontal circuits in alcoholic patients, impairing their ability to self-regulate mood, and rendering them more likely supplement their ability to modulate mood states with alcohol.

Table 4.1. Characteristics of Study Participants.

	Alcoholics (n = 15)	Controls (n = 15)
Males / females	8/7	8/7
Age	35.2 ± 7.34	33.3 ± 8.60
Years of Education	12.75 ± 1.60	16.8 ± 1.70
Average # drinking days/month	28.75 ± 4.33	8.0 ± 3.05
Average # drinks/drinking day	21.36 ± 8.85	1.88 ± 1.25
# comorbid drug abusers*	10	0
# patients with Axis I disorders	6	0
# patients with Axis II disorders	6	0

All categories differ significantly between groups ($p < 0.05$) except for age.

* Drugs of abuse included cocaine (7 patients), cannabis (4), sedatives (1), opiates (3), stimulants (1), and hallucinogens (5). All patients reported alcohol dependence as their primary complaint.

Table 4.2. Linear Contrasts between judgment and passive conditions within each group.

Comparison	Brain Region	*x*	*y*	*z*	volume	*t*-score	*p* value
Alcoholics: COGNITIVE > PASSIVE							
	L postcentral gyrus	-33	-32	43	6077	10.09	< .000001
	R medial frontal gyrus	1	57	18	3286	-8.125	< .000001
	R precuneus	34	-73	29	2933	7.548	< .00001
	R middle frontal gyrus	28	8	53	2328	7.372	< .00001
	L inferior frontal gyrus	-46	13	20	1702	7.89	< .00001
	L superior frontal gyrus	-27	9	50	1138	10.587	< .000001
	L precuneus	-30	-63	42	775	5.294	< .0001
Alcoholics: EMOTION > PASSIVE							
	L insula	-31	-30	12	10205	7.395	< .00001
	L posterior cingulate	-8	-72	10	5947	5.605	< .0001
	L postcentral gyrus	-38	-27	50	2610	6.386	< .0001
	R middle frontal gyrus	38	2	55	830	6.049	< .0001
	L fusiform	-17	-66	-13	827	5.179	< .001
	L insula	-28	9	-7	666	5.424	< .0001
	R middle occipital gyrus	36	-83	13	662	5.152	< .001
Controls: COGNITIVE > PASSIVE							
	L postcentral gyrus	-31	-37	45	3418	6.297	< .0001
	L parahippocampal gyrus	-26	-54	-2	1353	4.922	< .001
Controls: EMOTION > PASSIVE							
	L precuneus	-26	-55	49	3564	6.991	< .00001
	L parahippocampal gyrus	-26	-52	-1	961	6.056	< .0001
	R middle occipital gyrus	32	-77	19	817	4.68	< .001

Threshold is set at $p < 0.001$ uncorrected and a voxel threshold of at least 8 active voxels which yields a family-wise error rate correction of $p < 0.05$.
x, *y*, and *z* represent coordinates in the stereotactic atlas of Talairach and Tournoux.

Table 4.3. Comparisons between alcoholic patients and controls in each condition.

Comparison	Brain Region	*x*	*y*	*z*	volume	*t*-score	*p* value
Alcoholics > Controls: PASSIVE							
	R precuneus	9	-64	34	934	-5.474	< .0001
Alcoholics > Controls: COGNITIVE							
	L inferior frontal gyrus	-44	11	18	4526	9.034	< .000001
	L inferior frontal gyrus	-32	37	11	3482	7.625	< .00001
	R postcentral gyrus	31	-36	32	912	5.186	< .001
	L putamen	-21	9	-3	892	4.033	< .001
	L superior frontal gyrus	-9	29	43	859	4.736	< .001
	L insula	-49	10	5	520	6.674	< .00001
	L insula	-47	-38	19	740	6.839	< .00001
	L middle frontal gyrus	-35	9	59	586	5.303	< .0001
Alcoholics > Controls: EMOTION							
	L inferior frontal gyrus	-51	32	9	2887	5.456	< .0001
	L inferior frontal gyrus	-60	6	22	1972	5.24	< .001
	L putamen	-26	-5	2	1688	5.051	< .001
	R putamen	17	7	11	1582	4.83	< .001
	L postcentral gyrus	-60	-19	17	1530	5.846	< .0001
	L superior frontal gyrus	-8	32	43	1064	7.565	< .00001
	L insula	-44	10	4	743	4.852	< .001
	R medial frontal gyrus	35	5	57	644	6.881	< .00001
	L superior temporal gyrus	-50	-51	17	565	5.734	< .0001
	L thalamus	-11	-17	0	565	5.084	< .001

*Positive t-scores indicate regions where alcoholics show greater activation than controls; negative t-scores indicate greater activation in controls than alcoholic patients.
Threshold is set at $p < 0.001$ uncorrected and a voxel threshold of at least 8 active voxels which yields a family-wise error rate correction of $p < 0.05$.
x, y, and *z* represent coordinates in the stereotactic atlas of Talairach and Tournoux.

Table 4.4. Direct comparisons between alcoholic patients and controls (alcoholic > control), separated by emotional valence, for each condition.

Condition	Emotional Valence	Brain Region	Talairach coordinates *x*	*y*	*z*	Volume	*t*-score*	*p*-value
PB	Positive	R precuneus	13	-65	36	2040	-4.19	< .001
		L parahippocampal gyrus	-35	-11	-22	560	-4.58	< .0001
	Negative	L middle temporal gyrus	-53	-75	14	2928	3.77	< .001
		R cuneus	-5	-83	12	952	-4.13	< .001
		R superior frontal gyrus	29	57	-14	904	4.22	< .001
		R inferior frontal gyrus	61	11	18	752	4.56	< .001
		R middle temporal gyrus	37	-61	20	640	3.56	< .001
CB	Positive	L precentral gyrus	-49	-7	48	3032	4.43	< .001
		L middle frontal gyrus	-35	11	56	1864	4.65	< .0001
		R inferior parietal lobule	31	-39	32	1552	4.55	< .0001
		L inferior parietal lobule	-47	-63	48	1520	-4.80	< .0001
		R middle frontal gyrus	35	5	58	1032	4.30	< .001
	Negative	R middle frontal gyrus	31	3	50	6264	5.80	< .00001
		L inferior frontal gyrus	-49	13	2	3776	4.76	< .0001
		R medial frontal gyrus	13	31	40	3104	4.15	< .001
		L middle frontal gyrus	-35	47	12	1928	4.84	< .0001
		L putamen	-21	21	4	912	3.88	< .001
		R putamen	25	15	-2	888	3.98	< .001
EB	Positive	L precentral gyrus	-31	-13	58	4360	4.69	< .0001
		L postcental gyrus	-57	-21	18	4064	5.22	< .0001
		L superior temporal gyrus	-51	-53	16	2496	5.19	< .0001
		L thalamus	-11	-17	0	2008	4.56	< .0001
		R superior frontal gyrus	25	9	54	1768	4.95	< .0001
		L posterior cingulate	-17	-61	8	904	4.01	< .001
		R precentral gyrus	45	-3	44	904	3.90	< .001
		L putamen	-19	9	-4	800	3.90	< .001
	Negative	L cingulate gyrus	-3	23	42	5472	6.09	< .00001
		R middle frontal gyrus	33	3	56	3032	4.63	< .0001
		L insula	-37	-29	22	1760	5.56	< .0001
		L precentral gyrus	-49	-5	54	1232	3.81	< .001
		L superior temporal gyrus	-49	-51	16	1104	3.73	< .001
		R precentral gyrus	59	-1	40	936	4.77	< .0001
		L medial frontal gyrus	-13	41	20	640	5.30	< .0001

*Positive t-scores indicate regions where alcoholics show greater activation than controls; negative t-scores indicate greater activation in controls than alcoholic patients. Threshold is set at $p < 0.001$ uncorrected and a voxel threshold of at least 8 active voxels which yields a family-wise error rate correction of $p < 0.05$.

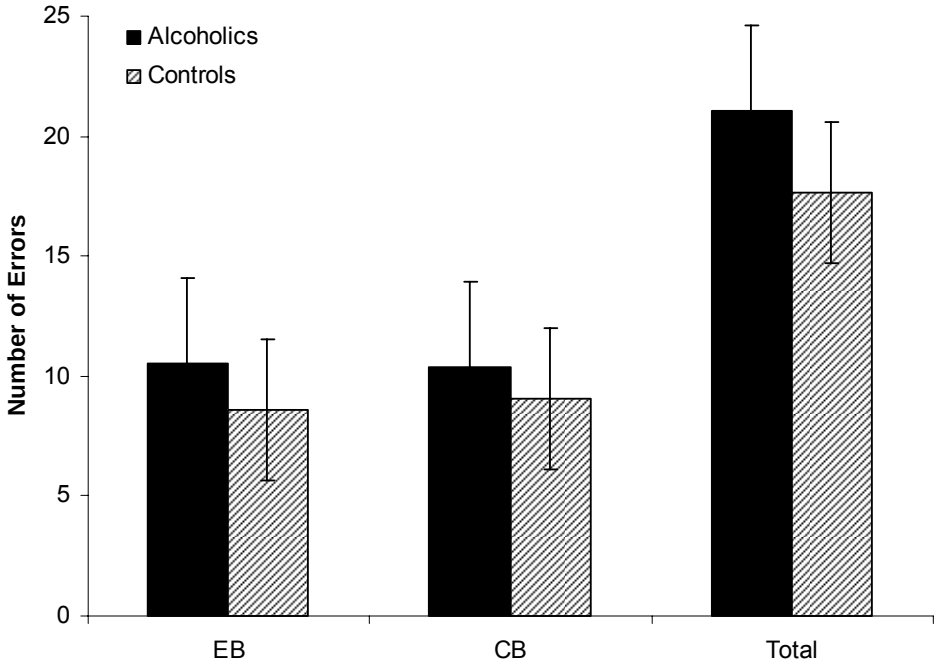


Fig 4.1. Errors in each group in each judgment condition. Although the alcoholics committed a greater number or errors in each condition, there was no significant difference in the number of errors committed by alcoholics and controls ($p > 0.05$). There was a total of 80 images (40 negative and 40 positive) in each condition. Error bars indicate SEM.

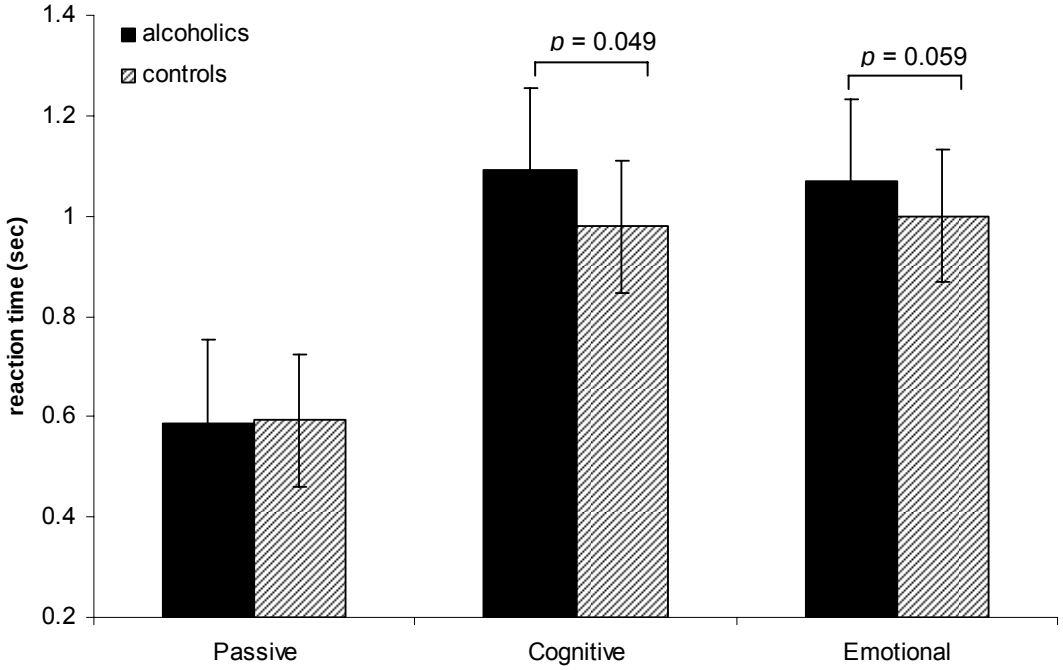


Fig 4.2. Reaction time in each group across conditions. There was no significant difference between alcoholics and controls in reaction time the passive condition, but differences reached trend-level significance in the cognitive and emotional blocks. Error bars indicate SEM.

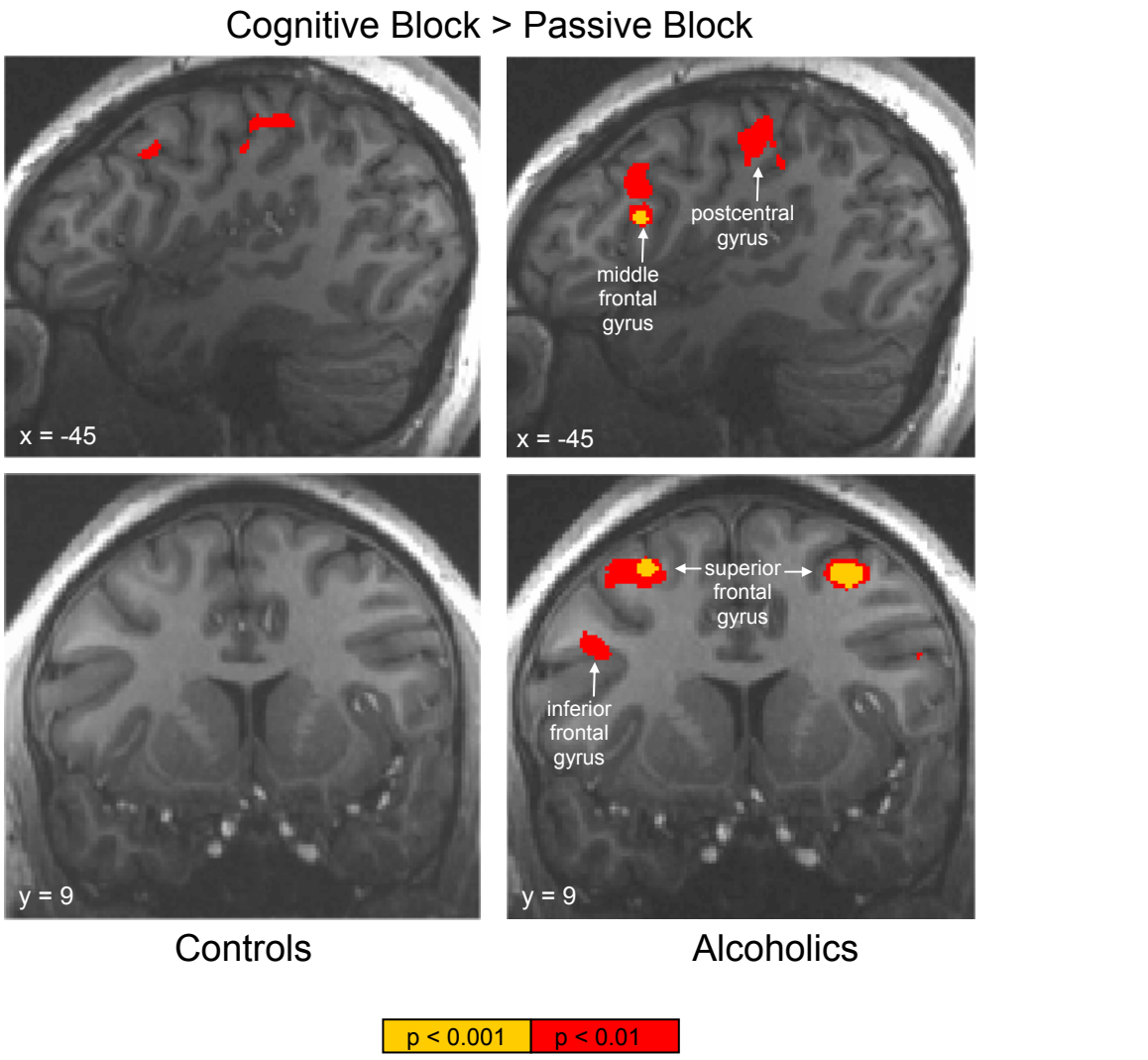


Fig 4.3. Linear contrasts brain regions showing greater activation to the CB than the PB in controls (left) and alcoholics (right). While controls had few regions that differed significantly in the CB and PB, alcoholics demonstrated greater activation in the CB relative to the PB in frontal and motor regions. Anatomical maps of *t* statistics were spatially normalized by warping to Talairach space and combined into a group map. Radiological convention is used to display left and right. The color map represents the *p*-value.

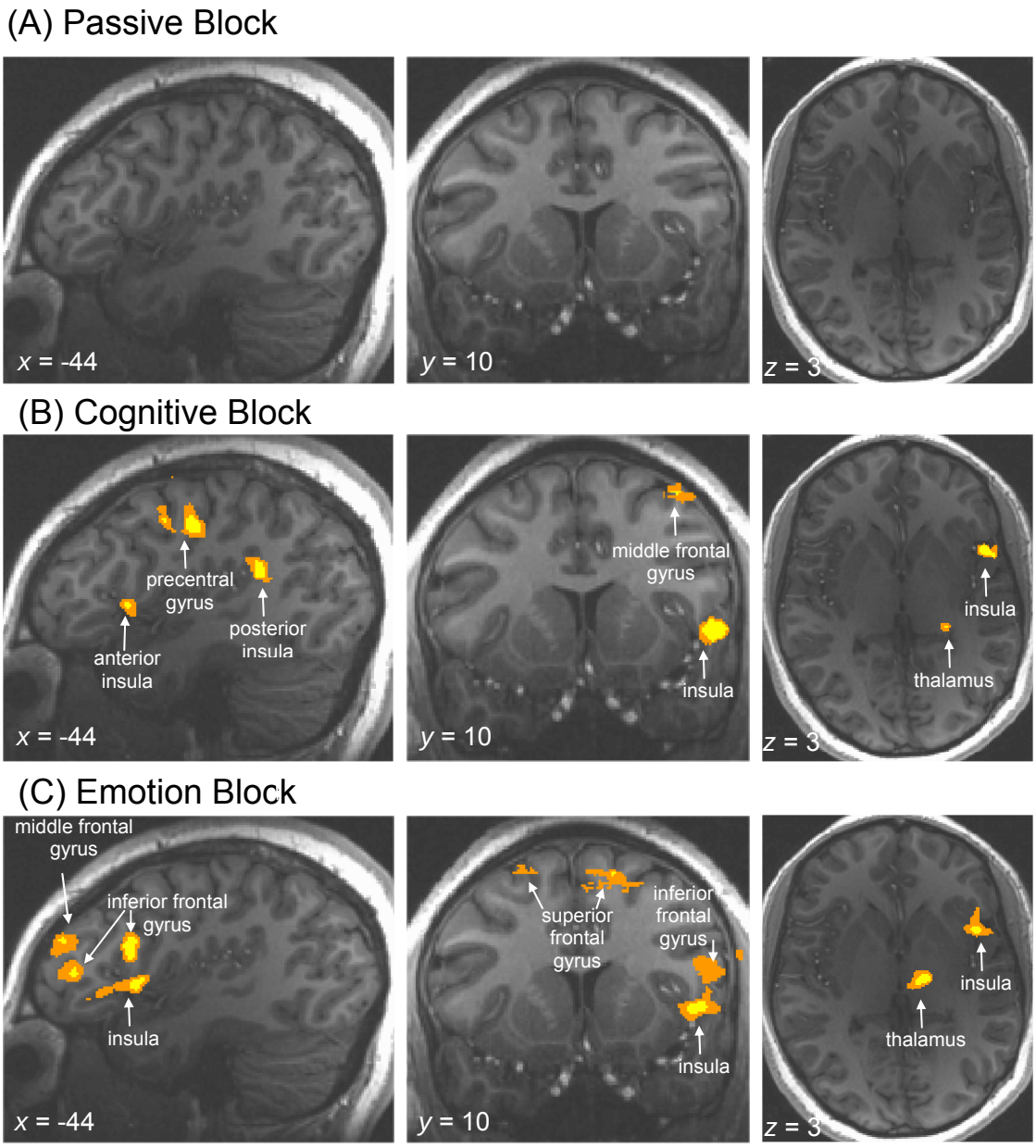


Fig 4.4. Difference in brain activity between alcohol-dependent patients and controls in the passive block (A), the cognitive block (B), and the emotional block (C). Anatomical maps of *t* statistics were spatially normalized by warping to Talairach space and combined into a group map. Radiological convention is used to display left and right. The color map represents the p-value where alcoholics demonstrated significantly greater activation than controls; in orange regions, $p < 0.005$, and in yellow regions, $p < 0.001$.

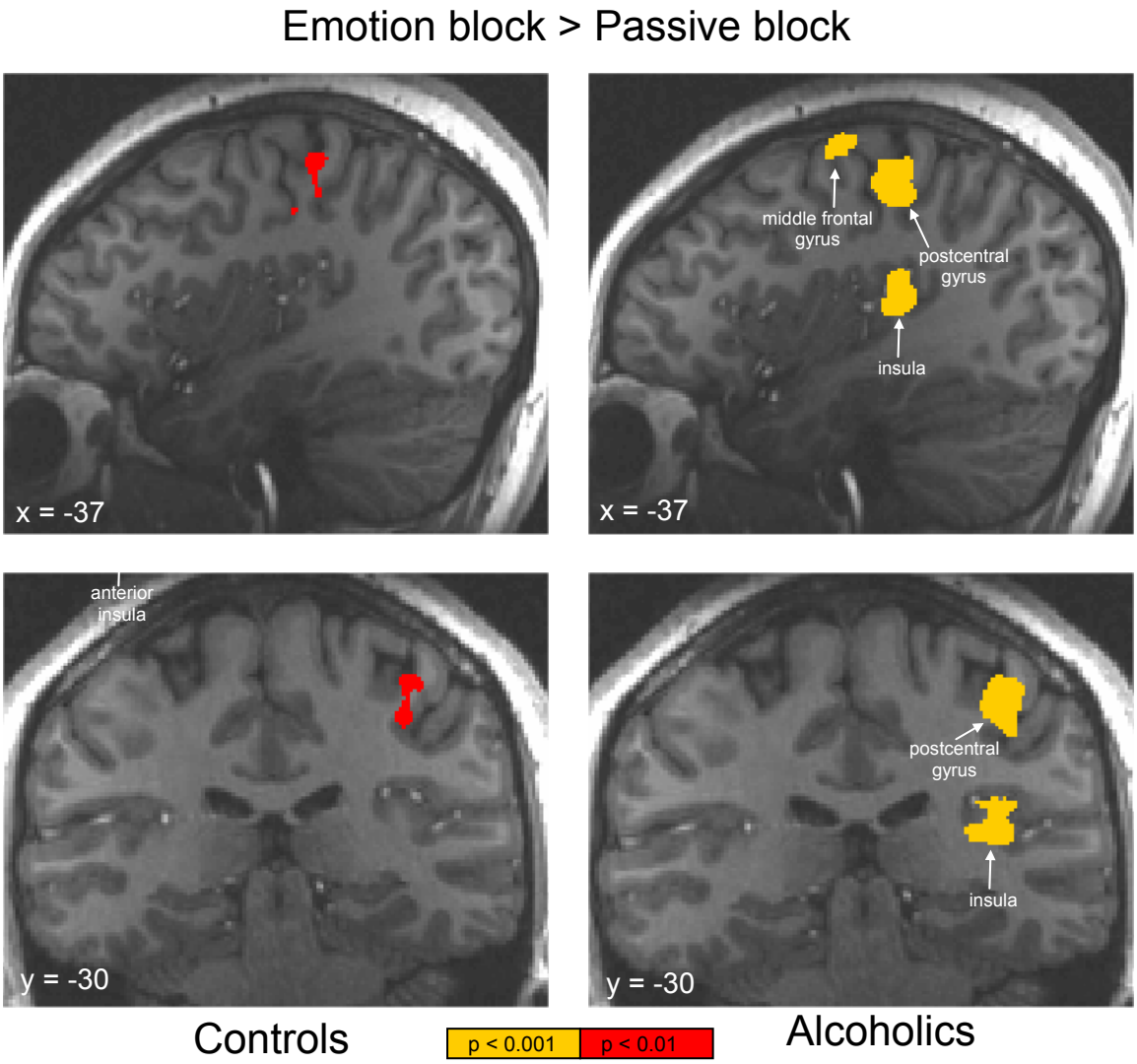


Fig 4.5. Linear contrasts brain regions showing greater activation to the EB than the PB in controls (left) and alcoholics (right). While controls had few regions that differed significantly in the EB and PB, alcoholics demonstrated greater activation in the EB relative to the PB in frontal, limbic, and motor regions. Anatomical maps of *t* statistics were spatially normalized by warping to Talairach space and combined into a group map. Radiological convention is used to display left and right. The color map represents the p-value.

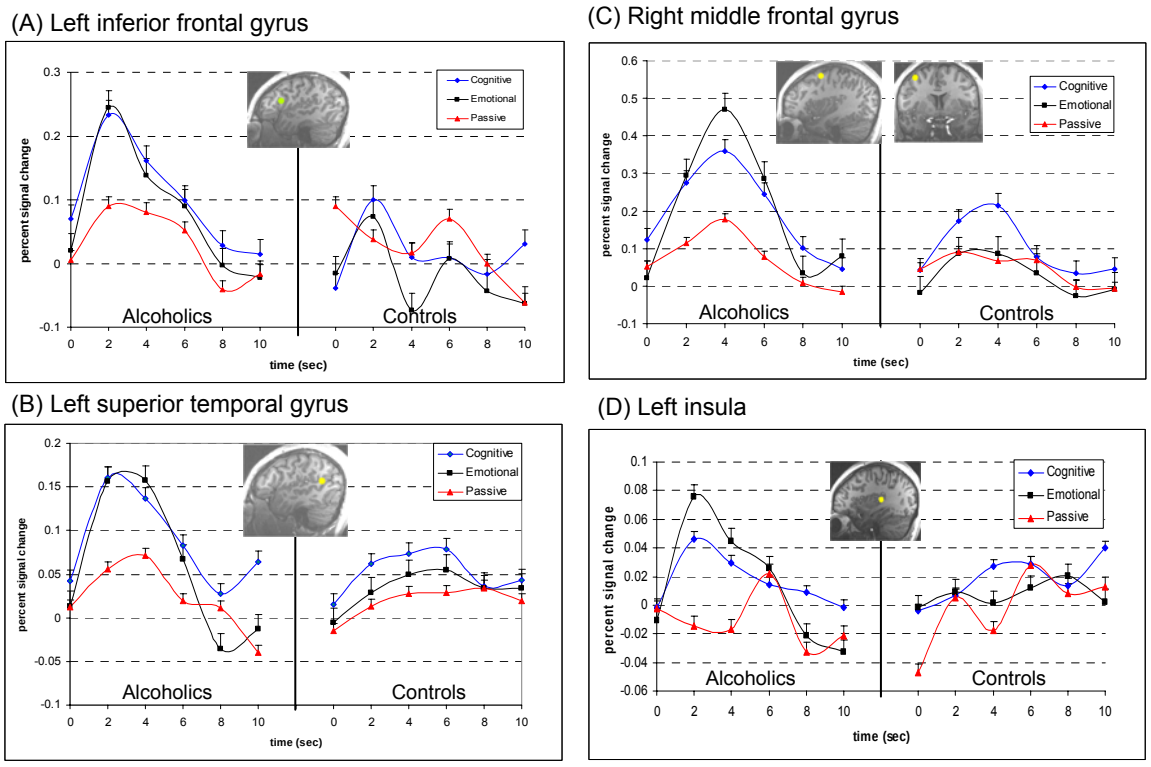


Fig 4.6. Time-course impulse response functions (IRF) in each volume of interest. See methods for description of IRF extraction. We performed multivariate ANOVAs in each VOI to test for differences between groups (alcoholics and controls), conditions (PB, EB, and CB), and emotional valences (negative and positive). In the inferior frontal gyrus (A), there was a main effect of group and condition; in the superior temporal gyrus (B) there was a main effect of group, condition, and a group x condition interaction; in the middle frontal gyrus (C) there was a significant effect of group, condition, and a group x condition interaction, and in the left insula (D), there was a main effect of group. See results for *F* and *p*-values. Error bars indicate SEM.

Chapter 5:

The Effect of Acute Alcohol Administration on Brain Response to Emotional Images

This experiment was designed to address a critical question in the field of alcoholism: Why do people drink alcohol? Alcohol-dependent patients who have deficits in emotional processing may choose to drink alcohol because of its ability to alter emotional states. Although people anecdotally report that alcohol induces euphoria or reduces anxiety, alcohol's effect on human brain circuits involved in reward and emotion has been explored only sparingly. In this study, we intravenously administered alcohol to social drinkers while brain response to visual threatening and non-threatening social stimuli was measured. We hypothesize that alcohol will activate the neural circuits involved in positive emotion (i.e. ventral striatum), and will decrease the neural response to fear-inducing stimuli in circuits involved in negative emotion (i.e. amygdala).[5]

[5] Most of this chapter is taken from an *in press* manuscript: Why we like to drink: An fMRI Study of the Rewarding and Anxiolytic Effects of Alcohol. **Gilman, J.**, Ramchandani, V., Bjork, J., Davis, M., and Hommer, D. *(Journal of Neuroscience, in press)*

5.1 Introduction

Behind only tobacco use and obesity, alcohol use is the third most common life style-related cause of death in the United States (Mokdad et al 2004). People like to drink alcohol because of its ability to alter emotional states. Alcohol induces euphoria, relaxation, and disinhibition, while reducing stress and anxiety. Consistent with human self-report, animal studies also suggest that alcohol produces a rewarding as well as an anxiolytic effect (Blanchard et al 1993; Coop et al 1990; Da Silva et al 2005; Spanagel et al 1995). Although its euphoric and stress reducing effects have been known for centuries and intuitively understood, how alcohol changes the function of human brain circuits has been explored only sparingly.

Where might alcohol recruit circuitry that regulates positive affect leading to euphoria? A critical area of interest is the ventral striatum (VS), which is recruited by reward-predictive stimuli (Bjork et al 2004b; Knutson et al 2001). The neural circuits that sense reward, predict reward, and initiate actions to acquire reward have been studied extensively. A reward can be defined as any stimulus that induces subjective feelings of pleasure, elicits exploratory or approach behavior, and increases the frequency and intensity of behaviors that lead to obtaining it (Shultz 2000). Rewarding stimuli consistently activate the mesocorticolimbic reward circuit, which includes the orbitofrontal cortex, amygdala, and striatum/nucleus accumbens, as well as the prefrontal cortex and the anterior cingulate gyrus (Knutson 2003; Sanfey 2003; Von Cramon 2003). A variety of primary and secondary rewards have been shown to activate this circuit, such as fruit juice and water (Berns 2001; O'Doherty 2002; Pagnoni 2002; McClure 2003), pleasant smells (Gottfriend 2002; Anderson 2003), and sexual stimuli (Arrow 2003), as well as conditioned rewards such as positive feedback and money (see Knutson 2005 for review).

The mesocorticolimbic reward circuit is also important in the development and maintenance of drug and alcohol addiction, and it has been hypothesized that all drugs of abuse share the ability to activate this circuit (Koob et al 1998). Functional magnetic resonance

imaging (fMRI) studies have shown activation in the striatum in response to cocaine (Breiter 1997) and nicotine (Stein 1998). While there have not yet been studies using fMRI to study acute alcohol administration, several researchers have used PET to demonstrate increased glucose metabolism or blood flow in response to ethanol. Porrino and colleagues used the quantitative autoradiographic 2-[^{14}C]deoxyglucose method to demonstrate increased glucose utilization in mesocorticolimbic and nigrostriatal dopamine circuits during the phase of rising blood alcohol concentrations (BACs) in rodents (Williams-Hemby and Porrino 1997). In humans, PET studies have demonstrated activations in the right prefrontal cortex (Tiihonen et al 1994), the left temporal cortex and left striatal regions (Wang 2000), and the bilateral thalamus (Boecker 1996), and preferentially in the ventral stiatum (Boileau et al 2003). These studies demonstrate the involvement of the striatal reward circuit in intoxication, but used oral alcohol administration, which is subject to extremely high interindividual variability of alcohol uptake even after controlling for sex and body weight (Ramchandani et al 1999). Shreckenberger and colleagues used intravenous (IV) alcohol infusion in a PET study using 18-FDG, and showed that IV alcohol stimulated the bilateral striatum and the frontal cortex, while deactivating the occipital cortex (Shreckenberger 2004), but no subjective measures of intoxication were collected in this study.

The striatum receives dopaminergic projections from substantia nigra and other midbrain nuclei such as the ventral tegmental area (Delgado 2007), but also has connections with limbic areas implicated in emotional processing (Groenewegen 1999). Thus, it is possible that the striatal response to ethanol could be influenced by emotional cues. The relationship between alcohol and emotion is extremely complex, and there is a large body of literature on various theories of how emotion and alcohol response can influence one another (see Stritzke et al 1996 for review), but thus far, no brain imaging studies have examined effects of alcohol intoxication on the processing of emotional stimuli. Participants in previous ethanol administration studies did not receive any sensory stimuli, which may not have accurately simulated natural intoxication conditions.

How might alcohol affect circuitry that governs negative affect to decrease anxiety? Alcohol-mediated anxiolysis may result from disruption of threat detection circuitry. The amygdala in particular is critical in an attention allocation circuit that is recruited by stimuli that signal the requirement for an immediate behavioral response, such as fight or flight (Fitzgerald et al 2006; LeDoux 2003). Alcohol intoxication increases incidence of aggression and social risk-taking (Corbin and Fromme 2002; Giancola et al 2002; Giancola and Zeichner 1997), perhaps by disrupting the amygdala-mediated differentiation between threatening and non-threatening stimuli. Since the amygdala is recruited as part of the mesocorticolimbic reward area, alcohol may influence amygdala activation. Stimuli that, under sober conditions, would not elicit amygdala activation (i.e. neutral, non-emotional stimuli) may do some under intoxicated conditions. This decreased differential response between fearful and non-emotional stimuli may increase approach while decreasing avoidance, which may facilitate social interaction.

The current study was designed to characterize the brain's response to alcohol intoxication and emotional stimuli, and is the first fMRI study to examine alcohol's acute pharmacological effects on the neural circuitry underlying emotion. This study extends previous research by (1) using intravenous (IV) alcohol to minimize individual variability in alcohol pharmacokinetics and to maintain a steady-state of brain alcohol exposure; (2) using fMRI to measure the blood oxygenation-level-dependent (BOLD) signal during alcohol administration, (3) presenting emotional facial stimuli, which allows the use of a general linear model (GLM) to examine main effects for alcohol and emotional cues as well as their interaction, and (4) collecting subjective measures of intoxication to correlate with BOLD signal.

5.2. Methods

Participants. Twelve community-recruited healthy social drinkers (7 women) participated in this study. All participants underwent a complete medical and psychiatric evaluation, including clinical laboratory, radiological and ECG examinations. Participants were excluded from the study if they had an abnormal physical exam or had laboratory values outside of normal ranges. Participants were given the Structured Clinical Interview for DSM-IV diagnoses and were excluded if they met criteria for alcohol or other substance dependency (excluding nicotine) at any time, or if they fulfilled DSM-IV criteria for a current axis-I psychiatric disorder. Participants were also excluded if they had never consumed at least two standard drinks of alcohol within one hour, or if they reported to have a "facial flushing" response to the consumption of alcohol. They drank an average of 1.9 days per week (SD = 1.1) and an average of 3.6 drinks per drinking day (SD = 1.2).

Participants were all right-handed, average age 26.5 years (SD = 5.6), and none had ever had a head injury requiring hospitalization. They were instructed not to take any prescribed, non-prescribed, or over-the-counter medications in the 14-day period prior to the study visits. Additionally, participants were asked to abstain from alcohol for at least 3 days prior to each study visit. A urine sample was obtained from each participant during each study visit for a urine drug screen and for a pregnancy test in females.

Alcohol Infusion Procedure. Alcohol was infused as a 6%v/v solution in saline. The infusion rates were based on a physiologically-based pharmacokinetic model for alcohol (Ramchandani et al 1999), consisting of an exponentially increasing infusion rate from the start of the infusion until the target breath alcohol concentration (BrAC) of 0.08 g% was reached at 15 min, followed by an exponentially decreasing infusion rate to maintain (or “clamp”) the BrAC at the target level. This infusion-rate profile was computed using individualized estimates of the model parameters, which are based on the participant’s height, weight, age and sex. This method has been used

successfully in several studies of the pharmacokinetics and pharmacological effects of alcohol in humans (Blekher et al 2002; Kwo et al 1998; Morzorati et al 2002; Ramchandani et al 1999; Ramchandani et al 2002; Ramchandani et al 2001).

Experimental Design. This study consisted of three infusion sessions given on separate days, separated by at least 3 days. On each study session, participants reported to the NIH Clinical Center Day Hospital, where BrAC levels were measured and a urine drug screen and, in women, a urine pregnancy test, were performed. An IV catheter was inserted in each forearm; one was used for the infusion of alcohol or saline and the other for the collection of blood samples.

The first study session (familiarization session) took place in the Clinical Center Day Hospital. Participants received an alcohol infusion over 45 min, to ensure that they tolerated the alcohol infusion without experiencing nausea or marked sedation, prior to undergoing the infusions in the fMRI scanner during the second and third sessions. Serial breathalyzer measurements were obtained every 3-5 minutes from the start of the infusion using the Alcotest 7410 handheld breathalyzer (Drager Safety Inc., CO), to ensure that the BrACs were within 0.01 g% of the target and to enable minor adjustments to the infusion rates to overcome errors in parameter estimation and experimental variability (O'Connor et al 2000; Ramchandani et al 1999). Subjective response to alcohol was measured using the Biphasic Alcohol Effects Scale (BAES) (Martin et al 1993) and the modified Drug Effects Questionnaire (DEQ), (de Wit and McCracken 1990) which were given before the beginning of the infusion and every 10-15 minutes during the infusion. Mood ratings were obtained before the start and at the end of the infusion using the Positive and Negative Affect Scale (PANAS) (Watson et al 1988). Blood samples (6 ml) were collected at three time-points: before the start of the infusion, and at 15 and 45 min after the start of the infusion. After the infusion was completed, BrAC measurements were taken every 30 min. Participants were sent home in a taxi cab when their BrAC dropped below 0.02 g%.

On the second and third study sessions, participants received the infusions in the scanner. One of these infusions was saline (placebo) and one was alcohol, given in a double-blind, randomized order during sessions 2 and 3. On these days, following IV catheter insertion, participants were placed in the scanner. A nurse was present in the scanning room throughout the infusion.

Structural scans were acquired as the infusion began. Target blood alcohol concentration (BAC) was expected to be achieved at 15 min, and emotional images were presented at 25 min as functional scans were acquired as described below. Participants were instructed to focus on the images, but no response was required. Blood samples (6 ml) were collected at three time-points: before the start of the infusion, at 15 min after the start of the infusion, and at 45 min, when the infusion ended. The DEQ was given at baseline, and before and after the set of images. Participants also completed the PANAS before and after the scan. The total duration of the infusion was 45 minutes, after which participants were escorted from the scanner and immediately given a breathalyzer test. They were then transported to the clinical unit, where BrAC measurements were taken every 30 min. Participants were sent home in a taxi cab when their BrAC dropped below 0.02 g%.

Stimuli. Visual images from a series of standardized emotional facial expression (EFE) images (Matsumoto 1988) were used in this study. Forty-five neutral and 45 fearful faces, as well as a non-emotional control crosshair condition that served as the inter-stimulus interval, were presented in an event-related design that lasted 8 min 30 sec. The stimuli were each presented for 2 sec, and the ISI ranged from 0-8 sec. All stimuli were presented using a Linux laptop computer with in-house stimulus delivery software. They were projected using an Epson MP 7200 LCD projector onto a screen placed at the foot of the MRI scanner bed and were viewed using a mirror mounted on the head coil.

fMRI acquisition. Imaging was performed using a 3 T General Electric MRI scanner with a 16-channel head coil. Thirty contiguous 5.0 mm thick axial slices were acquired (in-plane resolution

3.75 mm x 3.75 mm), providing whole-brain coverage including subcortical regions of interest such as the nucleus accumbens (NAcc), as well as the prefrontal cortex including the VMFC (ventromedial frontal cortex) and OFC (orbitofrontal cortex), together with limbic areas (amygdala), anterior cingulate and the paralimbic areas and thalamus. Whole-brain high-resolution coronal structural scans were collected using a T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) pulse sequence, which facilitated localization and co-registration of functional data (voxel size = 0.859 x 0.859 x 1.2 mm, matrix 256 x 256 x 124, repetition time (TR) = 100 ms, echo time (TE) = 12 ms, field of view (FOV) = 24 cm). Functional scans were acquired using a T2*-EPIRT sequence that measure changes in blood oxygen level dependent (BOLD) contrast (210 volumes, TR = 2s, TE = 30 ms, flip angle = 90, matrix 64 x 64, in-plane matrix = 128, FOV = 24 cm, slice thickness = 5 mm). BOLD images were collected during the presentation of the stimuli.

fMRI analysis. Analyses focused on changes in BOLD signal contrast (hereafter, activation) that occurred as the participants viewed the images following alcohol or placebo administration. Analyses were conducted using Analysis of Functional Neural Images (AFNI) software (Cox 1996). Echoplanar image volumes were preprocessed as follows: (1) voxel time series were interpolated to correct for non-simultaneous slice acquisition within each volume (using sinc interpolation and the most inferior slice as a reference). (2) Volumes were corrected for motion in three-dimensional space. Motion-correction estimates indicated that no participant's head moved >1.0 mm in any dimension from one volume acquisition to the next. We imposed a 6 mm full-width half-maximum (FWHM) smoothing kernel in the spatial domain. (3) A mask was created so that all of the background values outside of the brain were set to zero. This allowed the calculation of the percentage signal change in each voxel. Statistical maps were generated for each individual separately by linear contrasts between the regressors of interest. The regressors of interest were the neutral and fearful EFE faces. Preprocessed time series data for each individual were then analyzed by multiple regression, which allowed co-variation of variables related to head

motion. The regression model consisted of the orthogonal regressors of interest and six regressors of no interest modeling residual motion after volume registration. Regressors of interest were convolved with a gamma-variate function that modeled a prototypical hemodynamic response before inclusion in the regression model (Cohen 1997). Idealized signal time courses were time-locked to image onset.

Anatomical maps of *t* statistics were spatially normalized by warping to Talairach space (Talairach 1988) and combined into a group map. Next, a statistical map of the main effects of alcohol and facial emotion was computed by performing a voxel-wise ANOVA of the event-related β-coefficients calculated from the general linear model (using inputs of the regression model). In this three-factor mixed-model ANOVA, drug (alcohol or placebo) and emotion (fearful or neutral) were fixed factors, and subject was a random factor. Linear contrasts between the fearful and neutral conditions separately under the alcohol and placebo conditions (neutral: alcohol vs. placebo; and fearful: alcohol vs. placebo), as well as linear contrasts between the alcohol and placebo condition under each emotion type (alcohol: fearful vs. neutral; and placebo: fearful vs. neutral) were computed by performing voxel-wise *t*-tests between event-related β-coefficients of each stimulus type. When reporting ANOVA results, a family-wise error rate correction (using a Monte Carlo simulation) was applied to rule out false positives. When computing family-wise error-rate correction, statistical maps were resampled back into original voxel size. Clusters larger than 5 voxels at an individual voxel threshold level of $p < 0.001$ were considered significant. *T* statistics from the group maps were subsequently characterized by assessment of actual BOLD signal changes in volumes of interest.

Regions that have previously been implicated in either brain reward circuits (nucleus accumbens, putamen and caudate) or emotional-visual circuits (amygdala, fusiform gyrus, and lingual gyrus) were characterized with VOI analyses, in which time series signal data were analyzed. The VOIs were drawn as spheres with a radius of 5 mm, which was a small enough size to average signal data within the boundaries of small structures such as the NAcc and caudate,

which were of *a priori* focus, and also allowed us to investigate the source of effects that were driving significant activations due to alcohol, emotional valence, or interactions in other cortical regions *post-hoc*. Signal data were extracted from the time series as follows: (1) signal at each voxel was converted to a (percentage) deviation from the mean for that voxel across the entire time series, (2) signal was averaged by stimulus type and spatially translated into Talairach space, and (3) a mask was created consisting of the volume of interest through which each individual participant's data was extracted. These data were subject to analysis of variance using the percent signal change in each region as the dependent variable and alcohol (alcohol or placebo), emotion (fearful or neutral), and the interaction between them as the independent variables (package JMP-SAS; SAS Institute, Cary, North Carolina). The *p*-value for significance (two-tailed) was set at 0.05. In cases of significant interactions, post-hoc *t*-tests were performed to evaluate differences between the conditions.

Correlational Analysis. Coefficients of association were computed for the magnitude of change in the VOI measures versus subjective measures of intoxication, as measured by the DEQ. For these analyses, the VOI measure was a BOLD "change score," calculated as the average percent signal change to a stimulus class during the alcohol session minus the average percent signal change to the same stimulus class during the placebo session. A positive change score indicates that the participant exhibited a larger response during the alcohol condition, and a negative score indicates a larger response during the saline condition. Analysis was conducted using BOLD signal change under the neutral condition because this was the emotion type that demonstrated striatal activation.

5.3. Results

Self-reported alcohol effects

All participants tolerated the infusion without complications. The average BrAC reached during the familiarization session was 0.070 g% (SD = 0.008). All participants reported both stimulation and sedation effects on the BAES during this session (**fig 5.1**). We saw the expected subjective effects of alcohol on the DEQ, with significant increases in high, intoxication, "feeling effects," "liking" the drug, and "wanting more" of the drug, compared to baseline (**fig 5.2**). Participants reported peak ratings of intoxication and feeling high at 25 min after the start of the infusion.

During the scanning sessions, the average blood alcohol concentration was 0.0 g% on the placebo day, and 0.072 g% (SD = 0.009) at the end of the infusion on the alcohol day. Participants were asked to report subjective feelings of intoxication and high using the DEQ every 10 min during the scans. None of the participants reported feeling any alcohol effects on the placebo day, and during the alcohol day, they reported peak intoxication from 25-45 min after the start of the infusion (**fig 5.3**).

Participants did not differ significantly in self-report of positive or negative affect, measured by the PANAS, between the alcohol and the placebo day either before of after the scan (**fig 5.4**). On the alcohol day, there was no change in negative or positive affect from pre- to post-scan, but on the saline day, participants reported a decrease in positive affect from pre- to post-scan ($p = 0.003$).

Neural activity

To test for the main effects of the alcohol and the facial emotion type, as well as the interaction between them, we used a general linear model (GLM) where alcohol (alcohol or placebo) and facial emotion (fearful or neutral) were fixed factors, and subject was a random factor. We found a significant main effect of alcohol intoxication on activation of VS across facial emotion types (**table 5.1**; **fig 5.5A**). Activation was also significant in the right

parahippocampal gyrus, left precuneus, left anterior cingulate, and left superior temporal gyrus. Conversely, we found a significant main effect of facial emotion irrespective of alcohol administration in the right amygdala, bilateral lingual gyrus, left superior temporal gyrus, right superior temporal gyrus, and right anterior cingulate (**fig 5.5B**). Significant interactions between alcohol and facial emotion were seen in the several regions, including the left insula, right lingual gyrus, left nucleus accumbens, and bilateral middle frontal gyri (**fig. 5.5C**). These interactions were characterized in *post hoc* volume of interest analyses (**fig 5.7**).

During the placebo infusion, fearful faces (in contrast with neutral faces) activated the amygdala, insula, and parahippocampal gyrus, as well as visual processing areas, with no regions showing greater activations to the neutral compared to fearful faces (**table 5.2; fig. 5.6A**). In contrast, when participants were intoxicated, the fearful faces did not elicit a larger response than the neutral faces in any region.

Although we detected a main effect of alcohol intoxication in the striatum, this effect was primarily driven by the participants' reaction to neutral, but not fearful, stimuli. Neutral faces elicited ventral striatum activation when subjects were intoxicated, but not when they were sober (**fig. 5.6B**). Fearful faces elicited increased activation in the left putamen, but in a much smaller and more ventral region than in the neutral face condition.

Volume-of-interest analysis

We characterized BOLD signal changes in volumes of interest (VOIs) that have previously been implicated in either brain reward circuits (e.g. NAcc, putamen and caudate) or emotional-visual circuits (e.g. amygdala, fusiform gyrus, and lingual gyrus). Fearful faces elicited greater activation than neutral faces in the right-lateralized amygdala, fusiform gyrus, and lingual gyrus (**fig 5.7**). Alcohol main effects were not statistically significant in these regions (see **table 5.3** for values). The alcohol x facial emotion interaction effect on amygdalar BOLD signal change reached trend level significance ($p = 0.08$). Pairwise simple effect *t*-tests clarified this trend, demonstrating that whereas fearful faces activated amygdala significantly more than did neutral

faces ($p < .05$) during placebo infusion (as seen in both the time-series linear contrast maps, and in the extracted VOI data), while this effect was no longer significant ($p > .10$) during alcohol intoxication.

In the striatal VOIs, there were no main effects of emotion, but there were significant main effects of alcohol in the right NAcc, right caudate, right putamen, and left putamen, and significant interactions between alcohol and emotion in the left NAcc and the left caudate (**fig. 5.7**; see **table 5.3** for values). In these two regions, post-hoc one-way comparisons indicated significant differences between the alcohol and the placebo condition when participants viewed the neutral faces, but no differences during the fearful face condition (**fig. 5.8A**).

Association between self-reported intoxication and neural activity

There was a significant positive association between subjective ratings of intoxication and BOLD change scores in the neutral facial expression condition in the left NAcc ($r^2 = 0.467$, $p = 0.020$) and in the left caudate ($r^2 = 0.354$, $p = 0.045$). This indicates that participants who reported feeling more intoxicated showed a larger BOLD response to alcohol in these regions (**fig. 5.8B**). Stepwise multiple regression indicated no effect of actual BAC levels, gender, or pre-scan mood ratings of negative or positive affect on BOLD activation. There was no correlation between subjective intoxication ratings and BAC levels, probably as a result of the minimal inter-subject variability in BAC using our ethanol infusion method. We also did not find the session order to have a significant effect in any of our analyses.

5.4. Discussion

This study is the first to use fMRI to measure BOLD activation during intravenous alcohol infusion. The rapid IV administration of alcohol allowed us to achieve pharmacologically effective concentrations quickly, thus reducing acute adaptation and providing a clearer picture of alcohol's direct effects. The results confirmed our expectation that alcohol would robustly activate striatal reward areas in the brain, especially the ventral striatum. Activation in the left NAcc and left caudate increased relative to baseline signal in conjunction with subjective ratings of intoxication. These findings confirm PET data indicating increased glucose utilization during alcohol administration in striatum (Boileau et al 2003; Schreckenberger et al 2004; Wang et al 2000), and support Koob's hypothesis that all drugs of abuse activate the striatum (Koob 1992).

The anxiolytic effect of alcohol in visual-emotional brain areas

Despite alcohol's use in social settings, there have been no previous human imaging studies examining interactions between alcohol and emotional cues. A variety of non-imaging human studies have attempted to experimentally alter emotional states while administering alcohol or measuring alcohol intake (Stritzke et al 1996; Gabel et al 1980; Curtin et al 1998; Schroder and Perrine 2007), but these studies have not demonstrated a specific anxiolytic interaction between alcohol and emotional cues. Alcohol can have vastly different effects on emotion depending on factors such as the point on the blood alcohol concentration vs. time curve, the individual's drinking history, and the dose of alcohol consumed (Conrod et al 2001; Giancola and Zeichner 1997; King et al 2002; Levenson et al 1980; Lukas et al 1986; Turkkan et al 1988). Very few studies have systematically controlled these factors.

Our analysis found that visual and limbic brain regions were sensitive to the effects of alcohol. Emotional facial expressions activated higher order visual areas related to emotion--including lingual and superior temporal gyri as well as the affective division of the anterior cingulate as has been previously reported in studies exploring the effects of emotional stimuli on

brain activation (Devinsky et al 1995; Phillips et al 2003; Vuilleumier 2005). This result is consistent with previous findings that recruitment of these regions is enhanced by emotionally-valenced visual stimuli (Vuilleumier 2005). Importantly, the increased response to the fearful faces that we observed in the placebo condition was abolished in the alcohol condition. This suggests that alcohol may have attenuated the increased sensitivity of the visual system and limbic areas to emotionally threatening stimuli, and this may in part account for alcohol's anxiolytic effect. An alternative explanation involves alcohol's ability to increase activation in dopamine terminal regions, including amygdala, during the viewing of neutral faces. This increase in amygdala BOLD signal during neutral face presentation decreases the difference in amygdala activity between fearful and neutral faces, making the amygdala less able to act as a detector of threatening stimuli. This may not only lead to anxiolysis, but may also trigger an increase in both approach and aggression in some individuals.

The rewarding effects of alcohol in striatal brain areas

More than a third of the volume of the striatum was activated by alcohol across emotional conditions. The VS, particularly the NAcc, is critical in the reward system of the brain (Everitt and Wolf 2002; Koob and Nestler 1997; Robinson and Berridge 1993), and lesions in this brain region decrease the rewarding effects of many drugs of abuse (Di Chiara 2000). The reinforcing effects of alcohol most likely involve multiple neurotransmitter systems, including the dopaminergic, opioidergic, glutamatergic, GABAergic, and serotonergic systems. The increase in BOLD signal in the striatum may result from the increased firing rate of dopaminergic neurons secondary to the disinhibitory effect mediated through GABAergic interneurons and a decrease in glutamate-related potassium currents in the ventral tegmental area (Pierce and Kumaresan 2006). A recent review of animal pharmacological MRI suggests that NAcc dopamine release increases local BOLD signal via postsynaptic D1 receptor activation (Knutson and Gibbs 2007).

Consistent with previous studies that have shown significant inter-subject variability in subjective responses to alcohol at constant breath alcohol concentrations (Holdstock and de Wit

1998; Holdstock and de Wit 2001), we did not find a correlation between subjective ratings of intoxication and actual BACs. We also did not find a correlation between BOLD response and actual BAC, which was not entirely unexpected given that the infusion method was designed to minimize the inter-subject variability in BAC exposure. We did find a significant association between BOLD response in the NAcc and subjective perceptions of intoxication, suggesting that under conditions where the BAC is held constant, the intensity of the subjective feeling of intoxication is associated with VS activation.

In addition, our results suggest that alcohol-mediated striatal activation can be modulated by negative emotional stimuli. The participants' decreased striatal activation when viewing fearful faces suggests that the threatening stimuli may have attenuated the rewarding effects of the alcohol in the striatum, suggesting that context and environment influence the intensity of activation during intoxication.

Conclusions and future directions

This study demonstrates robust activation in response to intravenous alcohol infusion in the VS, an area that is critical in the acquisition and maintenance of addictive behavior. We were able to correlate striatal activation with subjective ratings of intoxication, indicating that the BOLD change in this area is directly related to an individual's subjective experience of alcohol's effects. Alcohol also modulates emotional processing in limbic and visual regions by decreasing the difference in activation between threatening and non-threatening stimuli, which may contribute to both the anxiolytic properties of alcohol, and to risky decision-making during intoxication. The data also indicate an interaction between alcohol and fearful emotional stimuli, such that fearful stimuli decrease striatal activation.

Although this study is underpowered to assess gender differences, future studies should examine whether alcohol has different effects on emotion in men and women, as previous research has shown that gender does influence emotional processing. For example, Klein and colleagues found no significant differences in activation between pleasant and unpleasant stimuli

in men, but significantly more activation to unpleasant than pleasant cues in women (Klein et al 2003). Furthermore, studies can assess gender differences in mood, striatal alcohol response, and any possible interactions between the subject's gender and the gender of the facial stimuli.

Future studies should also further explore the interaction between alcohol and emotion in alcohol-dependent patients and in individuals at risk for alcoholism. Previous studies have demonstrated differences between controls and alcohol-dependent patients (Heinz et al 2007; Salloum et al 2007), and between nonabusing adults with and without a family history of alcoholism (Glahn et al 2007), in brain regions involved in the processing of emotional stimuli. None of these studies, however, has examined differences among these groups in the effects of acute alcohol administration. These studies could enhance our understanding of how the neural correlates of intoxication and emotion contribute to addiction and risky behavior while intoxicated. In addition, it is possible that attenuation of the alcohol-mediated striatal BOLD response could be used as a surrogate marker for the clinical effectiveness of medications being developed for the treatment of alcoholism.

Table 5.1. Main effects of alcohol and emotion, and their interaction, on brain response.

Region	Talairach coordinates			Volume	*F* value	*p* value
	x	*y*	*z*	(mm^3)		
Alcohol						
Right Putamen	23	5	6	5272	47.83	< 0.0001
Left Putamen	-23	-1	0	3824	52.76	< 0.0001
Right Parahippocampal Gyrus	23	-45	-10	1568	23.04	< 0.001
Left Precuneus	-15	-43	40	1384	30.54	< 0.001
Left Anterior Cingulate	-17	25	24	696	22.19	< 0.001
Left Superior Temporal Gyrus	-57	-5	4	560	24.22	< 0.001
Emotion						
Left Lingual Gyrus	-23	-77	0	4160	30.88	< 0.001
Right Amygdala	17	-9	-9	3376	28.56	< 0.001
Left Superior Temporal Gyrus	-13	41	40	3208	24.82	< 0.001
Right Lingual Gyrus	17	-85	-4	3088	22.57	< 0.001
Right Superior Frontal Gyrus	13	45	36	776	22.91	< 0.001
Right Anterior Cingulate	3	31	-2	880	21.56	< 0.001
Interaction						
Left Insula	-41	-29	-2	8392	32.08	< 0.001
Right Lingual Gyrus	29	-79	-4	6528	42.02	< 0.0001
Left Middle Frontal Gyrus	-29	35	22	5368	50.44	< 0.0001
Right Posterior Cingulate	19	-63	16	2792	23.66	< 0.001
Right Inferior Frontal Gyrus	61	11	24	2168	30.78	< 0.001
Right Superior Temporal Gyrus	45	-19	0	2152	28.16	< 0.001
Right Middle Frontal Gyrus	31	41	20	1808	29.74	< 0.001
Left Posterior Cingulate	-11	-57	12	1272	30.41	< 0.001
Left Thalamus	-9	-25	0	1248	31.62	< 0.001
Left Middle Frontal Gyrus	-29	35	22	1168	50.44	< 0.0001
Left Nucleus Accumbens	-9	9	-8	633	34.11	< 0.001

Threshold is set at $p < 0.001$ uncorrected and a voxel threshold of at least 5 active voxels, which yields a family-wise error rate correction of $p < 0.05$.

Table 5.2. Brain activation by linear contrasts between each session and stimulus class.

Region	Talairach coordinates			Volume	*t*-score	*p* value
	x	*y*	*z*	(mm^3)		
Neutral: Alcohol > Placebo						
Left Putamen	-23	-7	10	27504	8.57	< 0.0001
Right Putamen	23	3	0	11472	7.63	< 0.0001
Right Superior Frontal Gyrus	23	35	32	5392	5.56	< 0.001
Right Posterior Cingulate	9	-49	24	12432	5.55	< 0.001
Left Lingual Gyrus	-25	-65	-6	2312	4.69	< 0.001
Left Cingulate Gyrus	-17	23	28	1992	4.75	< 0.001
Neutral: Placebo > Alcohol	No clusters detected					
Fearful: Alcohol > Placebo						
Left Putamen	-21	3	-4	448	7.53	< 0.0001
Right Superior Temporal Gyrus	49	-3	-10	456	5.19	< 0.001
Fearful: Placebo > Alcohol						
Left Lingual Gyrus	-13	-79	-14	320	6.17	< 0.0001
Left Parahippocampal Gyrus	-41	-33	-12	552	4.69	< 0.001
Placebo: Fearful > Neutral						
Left Amygdala	-21	-9	-14	3224	6.3	< 0.0001
Right Middle Frontal Gyrus	57	23	22	2784	6.05	< 0.0001
Left Parahippocampal Gyrus	-21	-27	-4	728	5.64	< 0.001
Right Lingual Gyrus	13	-91	1	7296	5.62	< 0.001
Left Lingual Gyrus	-19	-85	2	3056	5.55	< 0.001
Left Fusiform Gyrus	-37	-41	-12	5672	5.27	< 0.001
Right Anterior Cingulate	9	13	-8	952	5.23	< 0.001
Right Insula	41	-25	-2	5440	4.9	< 0.001
Right Inferior Frontal Gyrus	45	-3	24	1744	4.89	< 0.001
Left Medial Frontal Gyrus	-21	37	24	1760	4.65	< 0.001
Left Middle Frontal Gyrus	-47	19	42	880	4.61	< 0.001
Placebo: Neutral > Fearful	No clusters detected					
Alcohol: Fearful > Neutral	No clusters detected					
Alcohol: Neutral > Fearful						
Left Thalamus	-19	-15	10	4544	5.22	< 0.001

Threshold is set at $p < 0.001$ uncorrected and a voxel threshold of at least 5 active voxels, which yields a family-wise error rate correction of $p < 0.05$.

Table 5.3. ANOVA results of volume-of-interest analyses in striatal and visual-emotional brain regions.

Region	Alcohol	Emotion	Interaction		Alcohol	Emotion	Interaction
Striatal Regions				*Visual-Emotional Regions*			
Right NAcc (11, 10, -7)	$F = 6.09$ $p = 0.018$	NS	NS	Right amygdala (20, -5, -15)	NS	$F = 9.56$ $p = 0.004$	$F = 3.20$ $p = 0.083$
Left NAcc (-11, 10, -7)	$F = 8.63$ $p = 0.005$	NS	$F = 10.53$ $p = 0.002$	Left amygdala (-20, -5, -15)	NS	NS	NS
Right putamen (24, 5, 6)	$F = 14.27$ $p = 0.0007$	NS	NS	Right lingual gyrus (27, -79, -6)	NS	$F = 4.15$ $p = 0.049$	NS
Left putamen (-24, 5, 6)	$F = 15.11$ $p = 0.0004$	NS	NS	Left lingual gyrus (-27, -79, -6)	NS	NS	NS
Right caudate (13, 17, -3)	$F = 6.07$ $p = 0.019$	NS	NS	Right fusiform (29, -56, -8)	NS	$F = 4.3$ $p = 0.046$	$F = 3.86$ $p = 0.058$
Left caudate (-13, 17, -3)	$F = 7.64$ $p = 0.009$	NS	$F = 5.41$ $p = 0.027$	Left fusiform (-29, -56, -8)	NS	NS	NS

NS = non-significant

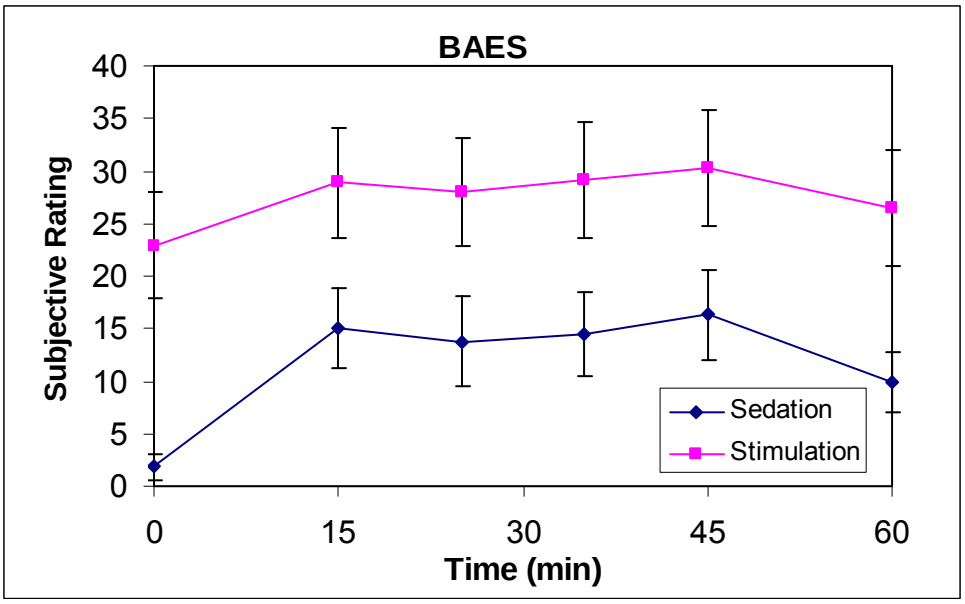


Fig 5.1. Biphasic alcohol effects scale. Participants reported significantly greater stimulation than sedation ($p = 0.01$). There was no significant effect across time from 15-60 minutes.

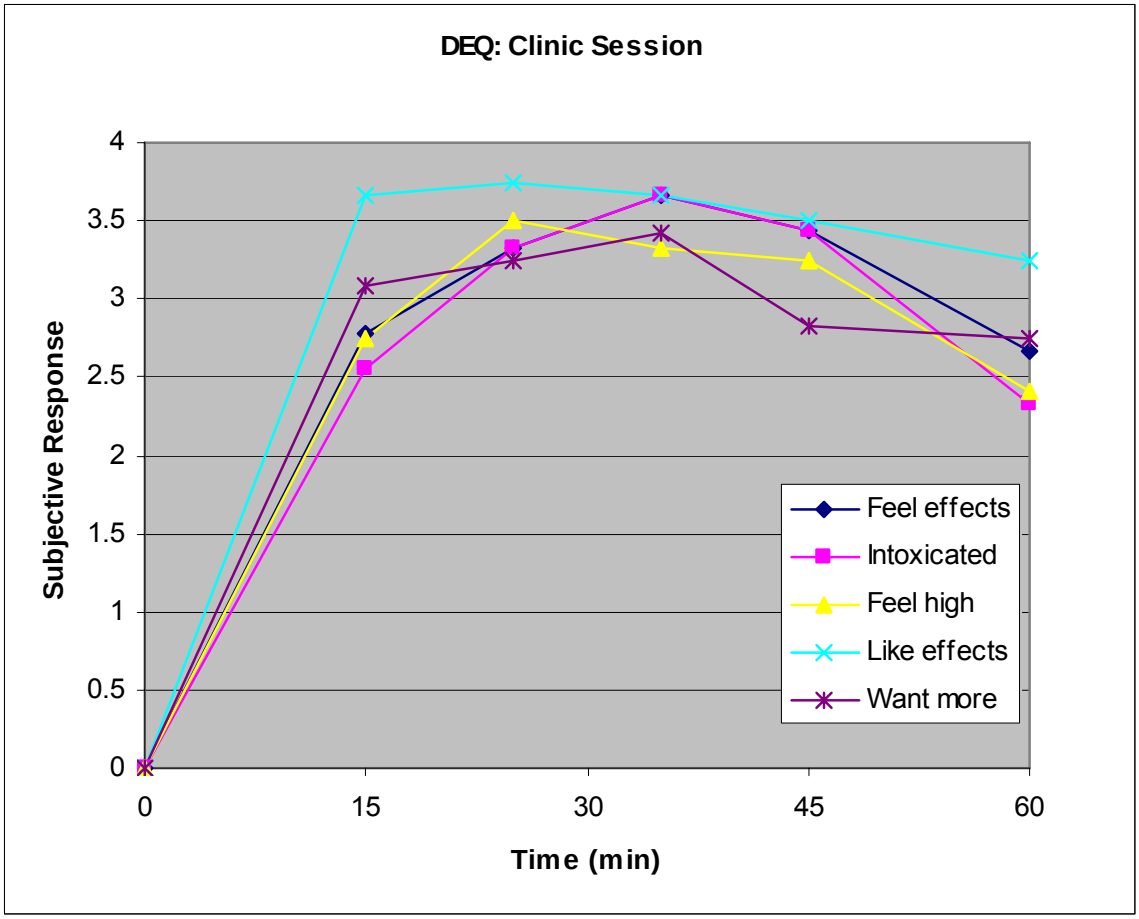


Fig 5.2. Drug effects questionnaire during the clinic session. Participants reported peak ratings of intoxication and feeling high 25 minutes after the start of the infusion. They reported peak values of "liking effects" and "feeling effects" at 15-35 minutes. There was no significant time effect across the period from 15-60 min.

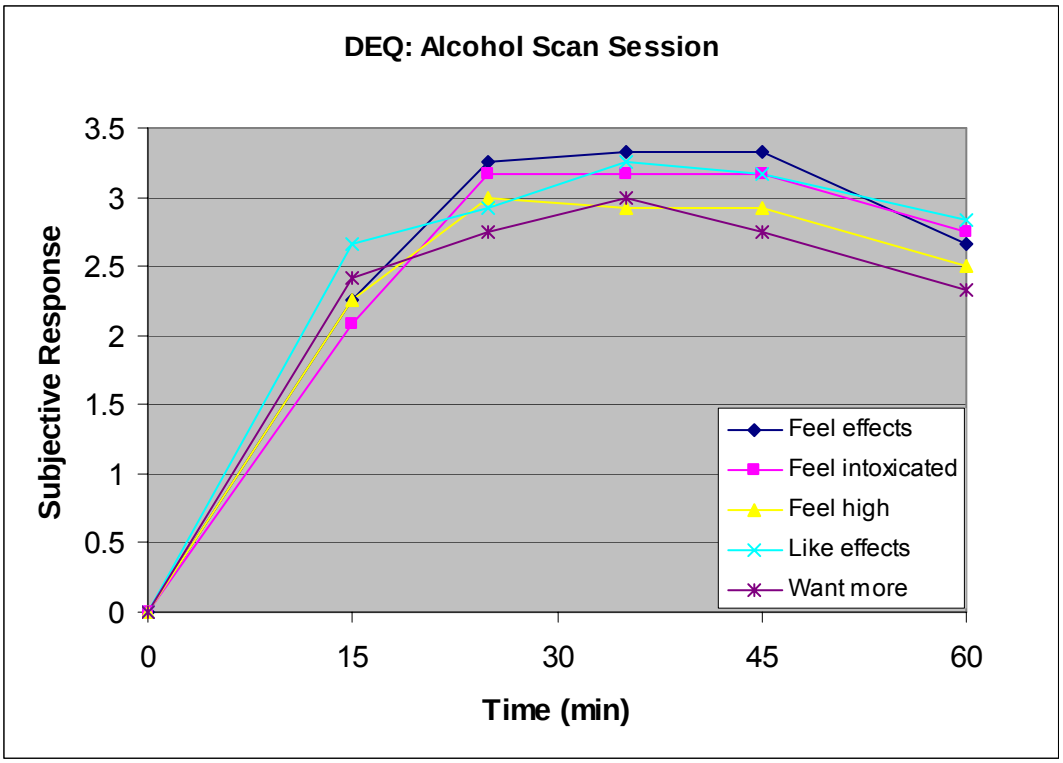


Fig 5.3. Drug effects questionnaire during the alcohol scan session. Participants reported peak subjective responses of the drug effects from 25-45 minutes after the start of the infusion.

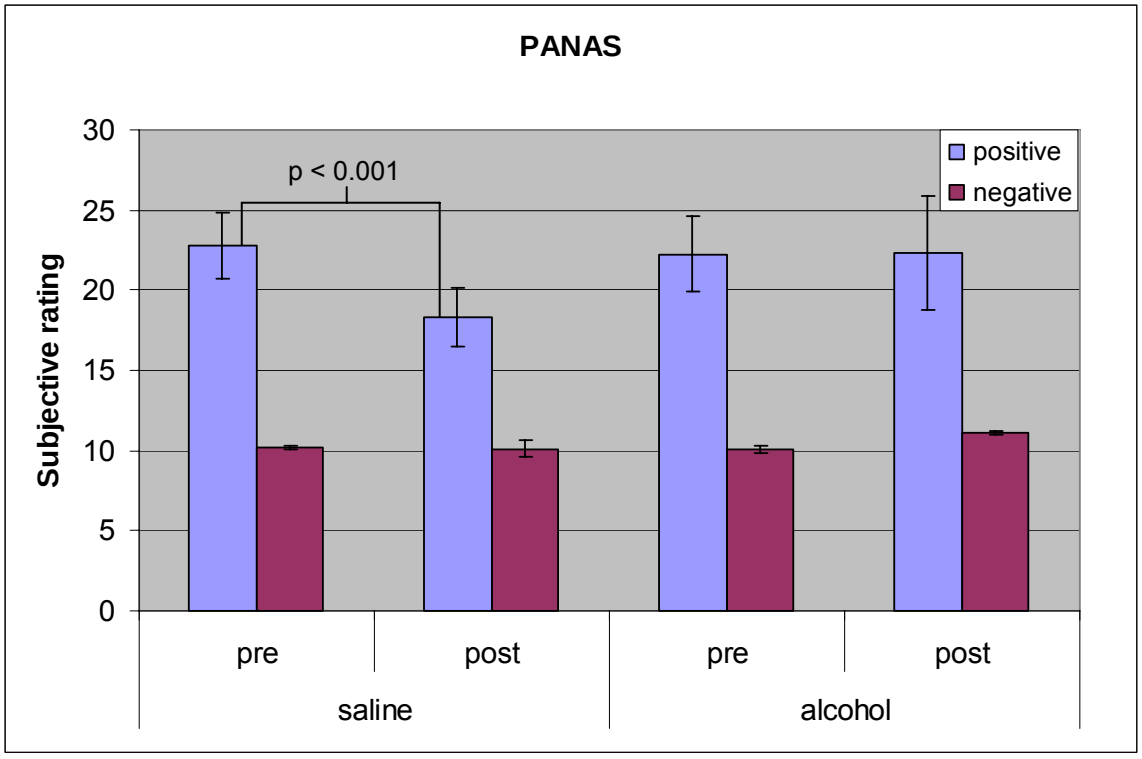


Fig. 5.4. Positive and negative affect scale. Participants did not differ significantly in self-report of positive or negative affect between the alcohol and the saline day either before of after the scan, but on the saline day, participants reported a decrease in positive affect from pre- to post-scan ($p = 0.0003$). Error bars indicate SEM.

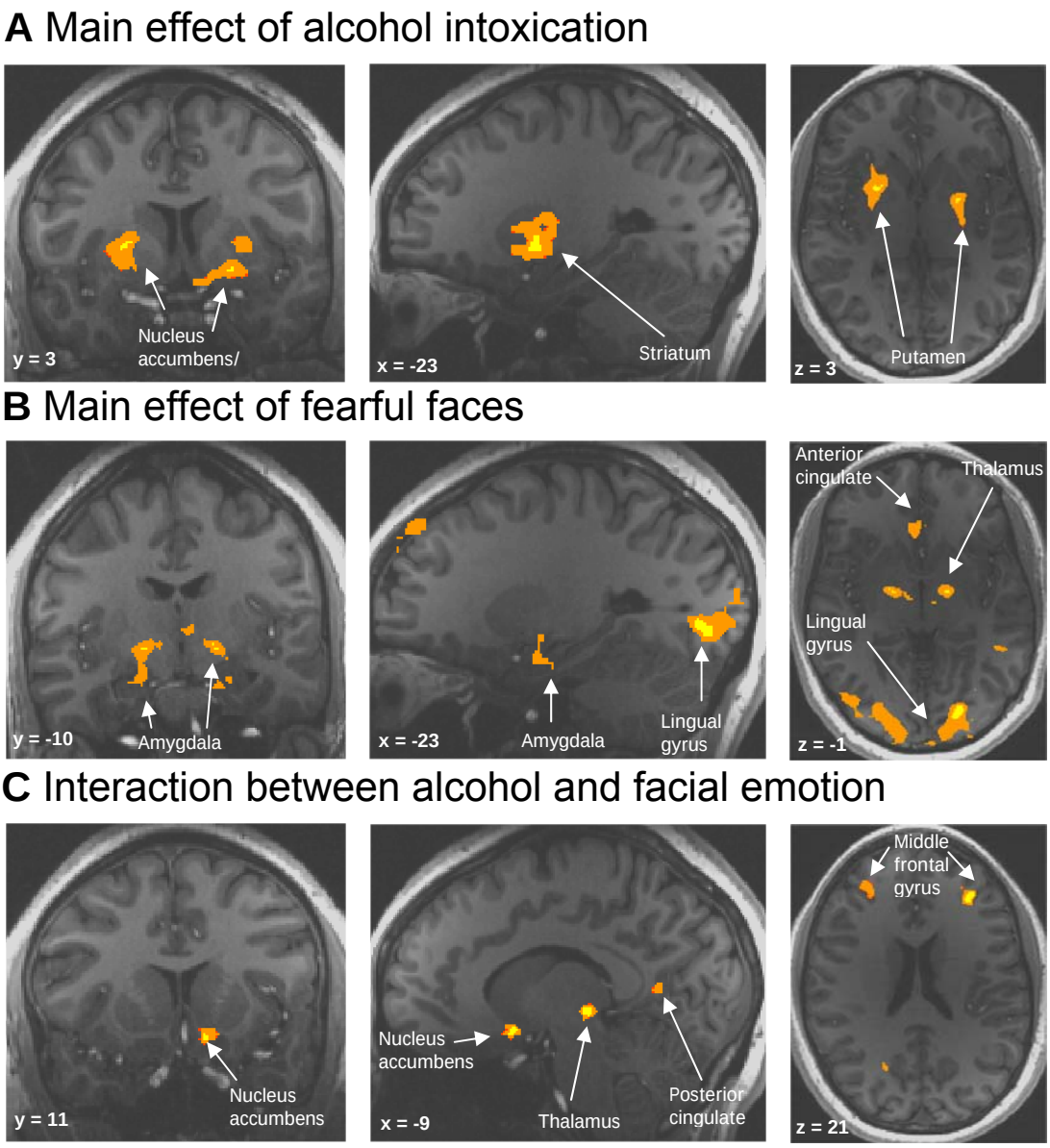


Fig. 5.5. Main effect of **(A)** alcohol, **(B)** fearful facial emotion, and **(C)** the interaction between them on regional brain activation. Anatomical maps of *t* statistics were spatially normalized by warping to Talairach space and combined into a group map. Radiological convention is used to display left and right. A statistical map of the main effects of alcohol and facial emotion was computed by performing a voxel-wise ANOVA of the event-related β-coefficients calculated from the general linear model. In this three-factor mixed-model ANOVA, alcohol (alcohol or

placebo) and emotion (fearful or neutral) were fixed factors, and subject was a random factor. Alcohol effects were seen primarily in striatal areas, while emotion effects were seen in limbic and visual processing areas. The color map represents the *t*-score; in orange regions, $p < 0.01$, and in yellow regions, $p < 0.001$. See **table 5.1** for values.

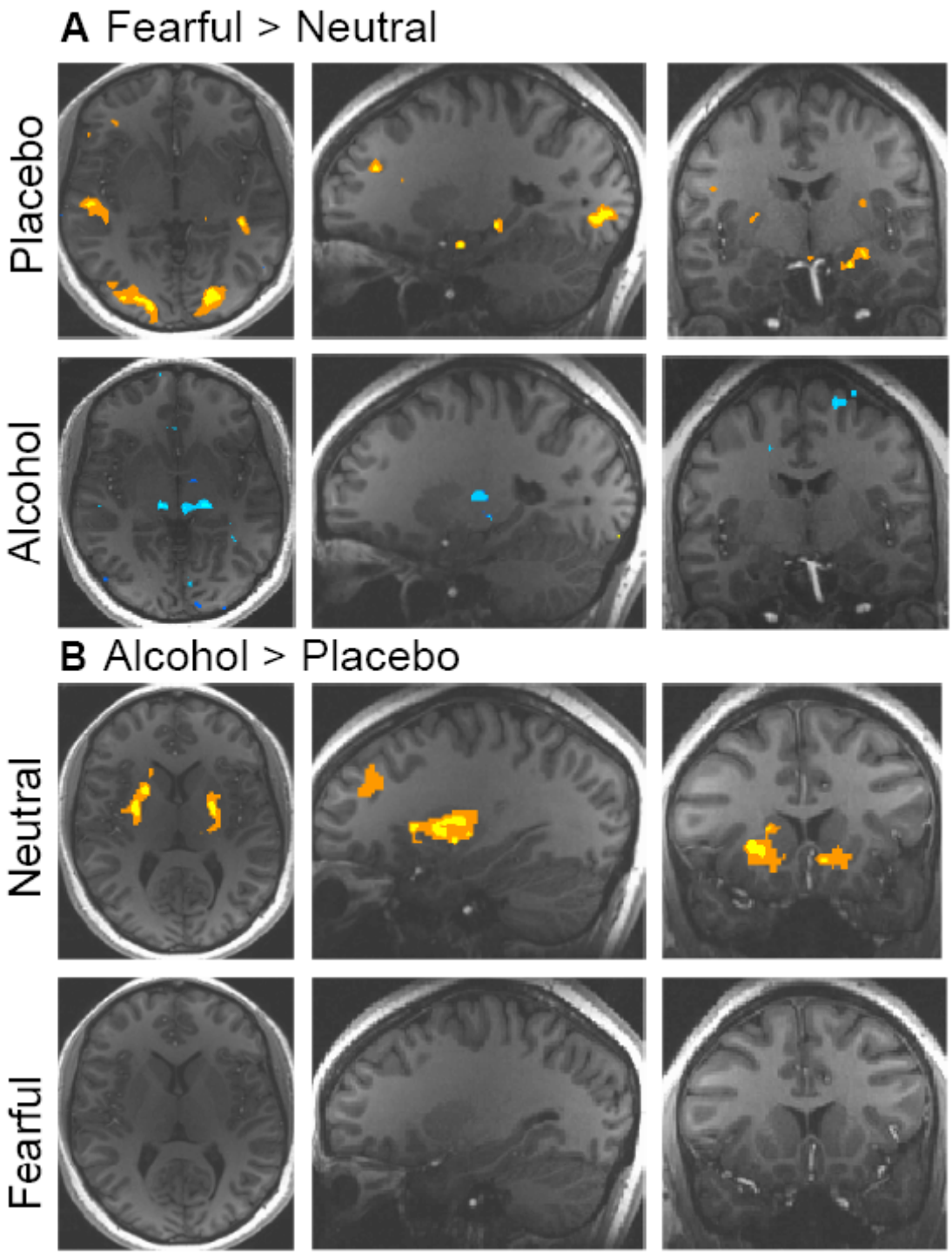


Fig. 5.6. Linear contrasts between the alcohol and placebo condition under each emotion type (alcohol: fearful > neutral; and placebo: fearful > neutral), as well as linear contrasts between the fearful and neutral conditions separately under the alcohol and placebo conditions (neutral:

alcohol > placebo; and fearful: alcohol > placebo). These contrasts were computed by performing voxel-wise *t*-tests between event-related β-coefficients of each stimulus type. Radiological convention is used to display left and right. **(A)** Linear contrast between fearful vs. neutral faces in the alcohol and the placebo condition. Increased activation to negative faces is shown in yellow/orange ($p < 0.01$), while increased activation to neutral faces is shown in blue ($p < 0.01$). **(B)** Linear contrast between alcohol and placebo in the fearful and neutral condition. Increased activation to alcohol is shown in yellow/orange ($p < 0.01$), while increased activation to placebo is shown in blue ($p < 0.01$). See **table 5.2** for values.

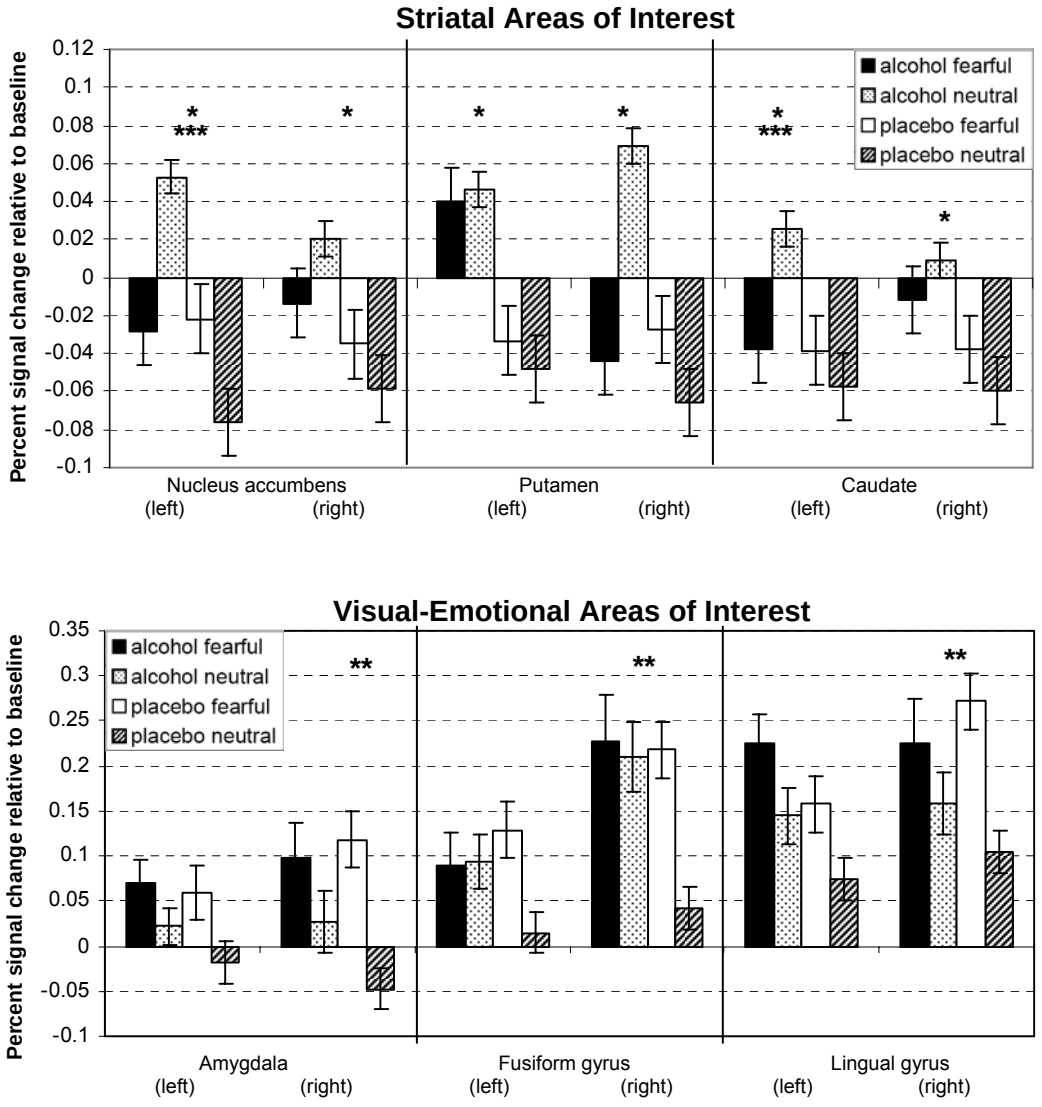


Fig 5.7. Percent signal change in volumes of interest in each condition. Values were entered into the GLM to test for main effects of alcohol, emotion, and an interaction. * indicates a significant main effect of alcohol; ** indicates a significant main effect of facial emotion; *** indicates a significant interaction between alcohol and emotion. Striatal areas of interest showed significant alcohol effects, while visual-emotional areas showed significant effects of emotion. In the visual-emotional areas, alcohol decreased the difference between response to fearful and neutral faces. See **table 5.3** for values. Error bars indicate SEM.

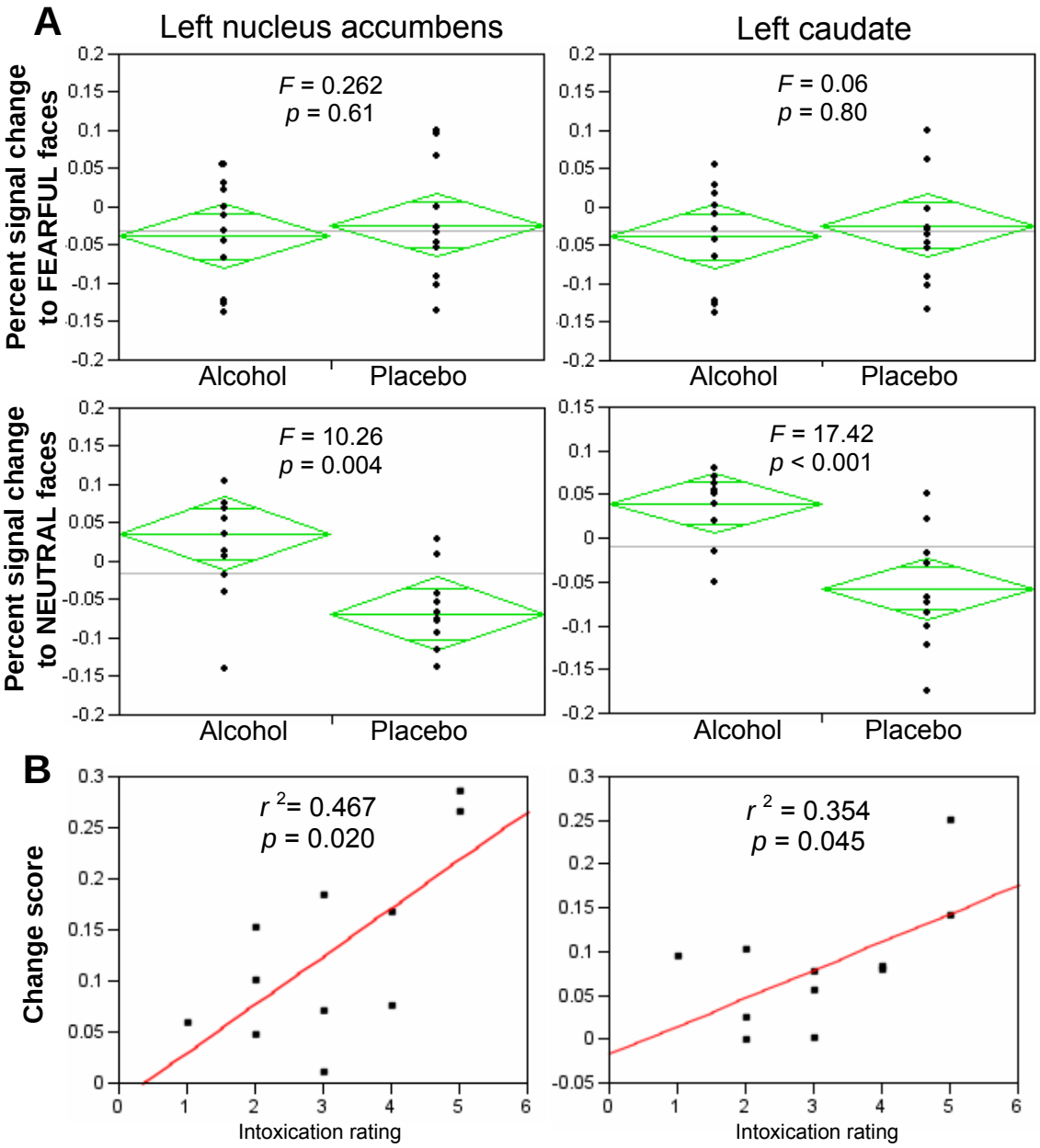


Fig. 5.8. Response to alcohol and neutral faces in the left nucleus accumbens and left caudate. **(A)** Where we observed significant interactions in the GLM (in the left NAcc and left caudate), we performed one-way ANOVAs. In these regions, there was a significant difference between the alcohol and the placebo condition when participants viewed the neutral faces, but no difference during the fearful face condition. **(B)** We ran a correlation between change scores, defined as percent signal change (to the neutral faces) during the alcohol session minus percent signal

change (to the neutral faces) during the placebo session, and intoxication ratings measured by the DEQ. There was a significant association between change score and intoxication in the left NAcc and the left caudate.

Chapter 6:

Are Differences Pre-existing? The Effect of Family History of Heavy Drinking on Brain Volume

Throughout this report, we have demonstrated that alcoholics have deficits in emotional processing, and that alcohol is a drug that affects emotional circuitry in the brain. A question that is not addressed, however, is if problems faced by alcoholics are a cause or an effect of the alcoholism itself. Though we have not studied emotional processing in alcoholics before they started drinking heavily, we can measure brain structures that are not affected by drinking. In this final study, we measured intracranial volume, a measure of maximal brain growth, in order to see if there were different structural characteristics between alcoholics with a family history of heavy drinking and those without a family history of drinking. We hypothesize that alcoholics with a family history of alcoholism may exhibit reduced brain growth and greater brain shrinkage than those alcoholics without a family history of heavy drinking[6]

[6]This chapter is taken from a published manuscript: Parental Alcohol Use and Brain Volumes in Early and Late-Onset Alcoholics. **Gilman, J.**, Bjork, J., Hommer D. *Biological Psychiatry*, 2007, Sep 15;62 (6):607-15.

6.1. Introduction

Children of alcoholics (COAs) are at greater risk of developing alcoholism than children from nonalcoholic families (Cotton 1979; Devor and Cloninger 1989; Sher 1991). Many factors contribute to this increased risk. In addition to inheriting genetic predisposition, COAs may suffer from both biological and psychological injury, stemming from poor diets, inadequate psychological support, unstable parental relationships, and gestational alcohol exposure due to maternal alcohol use, all of which could contribute to the development of alcoholism (Carrion et al 2001; De Bellis et al 1999; Rosso 1990; Welch-Carre 2005), in addition to social/environmental factors concerning abnormal alcohol use. However, except in the case of fetal alcohol syndrome (FAS), direct physical evidence for the effects of the putative genetic and environmental factors mediating the family transmission of alcoholism is lacking.

Many studies have shown that alcohol-dependent men and women have smaller brain volumes than their non-alcohol-dependent cohorts (Bjork et al 2003; Jernigan et al 1991; Pfefferbaum et al 1992), but an effect of family history of heavy drinking on brain volume in alcoholism has not been demonstrated. It is widely believed that most of the difference in brain volume between alcoholics and non-alcoholics is due to ethanol neurotoxicity which causes the alcoholic's brain to shrink with aging to a greater extent than the non-alcoholic's. If this is true then a family history of heavy drinking could only contribute to differences in brain volume between alcoholics and non-alcoholics by altering an individual's vulnerability to ethanol neurotoxicity or by causing alcoholics with a family history of heavy drinking to drink more than alcoholics without a family history. However, it is not clear that the difference in brain volume between alcoholics and non-alcoholics is due exclusively to ethanol neurotoxicity.

We and others have reported that alcoholics have smaller intra-cranial volumes (ICVs) than non-alcoholics (Bjork et al 2003; Cardenas et al 2005; Hommer 2003). These differences are around 2.5 % but they do not reach statistical significance. The small difference in ICV we

observed between alcoholics and non-alcoholics suggested that there could be a subgroup of alcoholics, such as COAs, with considerably smaller ICV. Unlike brain volume itself, ICV is a valid measure of brain growth because it is determined by skull growth, which occurs as the brain, meninges, and cerebrospinal fluid space expands to their maximal size around puberty (Carmichael 1990). ICV does not change as a function of neurodegeneration or aging like brain volume (Jenkins et al 2000), and therefore is a useful estimate of the lifetime maximum volume of the brain (Blatter et al 1995) . Though ICV is highly heritable, it may also be influenced by environmental conditions (Baare et al 2001), particularly when the environment is not ideal. There have been several animal studies demonstrating gestational exposure to ethanol causes decreased size of craniofacial structures (Edwards and Dow-Edwards 1991), and a small head size is one of the diagnostic criteria for FAS (Mattson et al 1996; Roebuck et al 1998). Some COAs who are not formally diagnosed with FAS may have fetal alcohol effects which are more subtle and may include slight reductions in skull and brain size

In this study, we used T1-weighted magnetic resonance imaging to measure ICV, cerebral volume, white and gray matter volume in both healthy controls and in adult treatment-seeking alcoholics with and without a positive family history (FH) of heavy drinking. We also further analyzed ICV, as well as soft tissue volumes, within the FH positive alcoholics as a function of which parent was a heavy drinker (neither, mother, father, or both) in order to determine if the alcohol use of each parent had a differential influence on brain growth and development. A study in rats demonstrated that even in alcohol-treated males who sired offspring, there was a significant increase in the number of "runts," or smaller than average offspring, at birth compared to those sired by non-alcohol-treated males (Abel 1993). We therefore hypothesize that a positive FH, even one limited to fathers alone, would be associated with smaller ICV, but those alcoholics with a maternal FH of heavy drinking would be most severely affected.

In addition to premorbid differences in brain growth as indexed by ICV, we also examined whether a family history of heavy alcohol use was independently related to the amount of brain shrinkage which occurs throughout adulthood. A previous study by Cardenas et al (2005) found a positive FH to be protective against brain shrinkage in heavy drinkers. In our study, brain shrinkage was be inferred from the ratio of cerebral volume to total ICV. If FH does affect ICV, then the smaller absolute brain volumes observed in alcoholics may be a result of either greater brain shrinkage with age, smaller maximal brain growth or both. Calculating a ratio of brain volume to ICV allows us to independently measure the contribution FH makes to brain shrinkage as well as brain growth.

Finally, many studies have shown a weak but consistent correlation between brain size and IQ (e.g. (Andreasen et al 1993; De Bellis et al 1999; Willerman 1991) and several studies indicate that COAs tend to do more poorly academically than control children (Ervin et al 1984), particularly in verbal skill (Gabrielli and Mednick 1983) . These cognitive/reasoning deficits may be related to a reduction in brain size in COAs. If a positive FH is correlated with decreased brain sizes and general neurodevelopmental deficits, we would observe lower IQ scores in FH positive alcoholics. We conducted IQ tests in order to see if ICV and a positive FH are accurate predictors of intelligence.

6.2. Methods

Participants

Alcohol-dependent patients (n = 231) were recruited from among all the patients consecutively admitted to the National Institute of Alcohol Abuse and Alcoholism (NIAAA) inpatient unit at the Clinical Center of the National Institutes of Health in Bethesda, MD between January 1995 and September 2004. Most patients lived in Montgomery County, MD, or the greater Washington, DC area. All participants were interviewed using the Structured Clinical

Interview for the Diagnostic and Statistical Manual of Mental Disorders. Information on recent and chronic alcohol use was obtained from structured research questionnaires. All subjects provided written informed consent to participate in the study which was approved by the NIAAA Institutional Review Board.

All alcoholic patients met DSM-III-R criteria for alcohol dependence. We excluded patients who met the criteria for alcohol abuse but not alcohol dependence, as well as those who had a history of delirium tremens or gross neurological disorders. In addition, we excluded patients who had an IQ less than 80 or who demonstrated signs of dementia or Korsakoff's disease. Participants were not thiamine deficient at admission, and none of the subjects had a history of head injury requiring hospitalization. Patients were scanned three weeks after admission, or if they had been transferred from another hospital, at least three weeks from the last alcohol use.

Family history was assessed by administering an interviewer-completed Lifetime Drinking questionnaire after the patient had undergone several weeks of group therapy focused on alcoholism. The family history of the participant was determined through the use of a rating instrument with six categories ranging from "does not drink" to "alcoholic." If a patient rated his or her biological father or mother as: a "heavy drinker," "problem drinker," or "alcoholic," the patient was considered to have a positive FH. We further subdivided patients according to age of onset of alcohol-dependence. Age of onset of alcoholism was defined as the age at which the patient first consumed 90 drinks in a one-month period. Early onset alcohol-dependent patients (EOAs; n = 129) had an age of onset of alcoholism between the ages of 13-25 years, and late onset alcohol-dependent (LOAs; n = 102) had an age of onset of alcoholism greater than 25 years of age. Average quantity (average number of drinks daily per drinking day) and frequency (number of drinking days per month) were also calculated over the six month period preceding admission. Years of heavy drinking was defined as the cumulative total contiguous or noncontiguous months during which the subject drank 90 drinks per month (note: since subjects

often maintain this high a level of alcohol use for at least 12 consecutive months, months were summed into years). Patients were considered comorbid substance abusers if they met DSM-III or DSM-IV criteria for drug abuse or dependence with a substance other than alcohol at some point in their life.

Healthy community-recruited male and female participants with no history of significant medical illness or psychiatric disorders were included for comparison (n = 114). Most control participants were also drawn from the Montgomery County, MD and greater Washington DC area. None of the control subjects reported a positive FH for alcoholism. All participants were assessed with the Structured Clinical Interview for either DSM- III-R or DSM-IV, which confirmed that each patient met criteria for alcohol dependence and that no comparison subject met criteria for a psychiatric disorder.

Magnetic resonance imaging scan acquisition and analysis

Participants were scanned with 1.5 T MRI (GE Medical Systems, Milwaukee, Wisconsin) using a fast spoiled-GRASS (FSPGR) sequence. A gapless series of high contrast 2 mm thick T1-weighted coronal images (repetition time, 25 msec, inversion time, 5 msec and echo time, 16 msec) was obtained. Images were acquired using a 256 by 256 matrix with a 240 by 240-mm field of view. Each volumetric brain consisted of 124 coronal slices with voxel size of 0.9375 by 0.9375 by 2.0 mm.

Intracranial tissue margins were marked manually on coronal sections with a hand-driven cursor. The ICV included the cerebrum and cerebrospinal fluid (CSF) spaces covering the cortex, but excluded the cerebellum and CSF of the posterior fossa. Inter-rater reliability for manual identification of the ICV of 10 randomly selected MRI volumes was high (intra-class correlation = .97). Next, brain tissue was automatically segmented into gray matter, white matter, sulcal CSF, and ventricular CSF using a previously described computerized method (Momenan 1997) that used voxel intensity to perform a K-means clustering procedure. Cerebral brain volume was

calculated by adding the white and gray matter volumes. Brain shrinkage was inferred by calculating the ratio of cerebral volume, gray volume, and white volume to total ICV.

Intelligence (IQ)

IQ was estimated using the WAIS-R vocabulary and block design tests (Wechsler 1981). IQ data was available for 203 alcoholic participants. The vocabulary test measured verbal intelligence, and the block design tested visuospatial abilities by requiring the subject to create geometric designs using blocks. These two subtests have previously been used as a "short-form" of the WAIS-R to estimate IQ (Silverstein 1983) and results of the short form significantly correlate with scores of the Full Scale test (Silverstein 1985). Age-corrected scaled scores were used to calculate estimated IQs.

Statistical Analyses

Data distributions were examined for normality. We used a general linear model (GLM) to examine the independent variables of sex, height, age, family history, age of onset of alcoholism, and all possible interactions on the dependent variables of ICV, brain volumes, and brain shrinkage as measured by the brain volume to ICV ratio (package JMP-SAS, SAS Institute; Cary, NC). We also used a GLM to test the independent variables of ICV, level of education, age, family history, and age of onset, as well as all interactions, on IQ scores. When an interaction was observed, we conducted post-hoc simple-effects analyses using a Students t-test. All significance testing was two-tailed with alpha = 0.05. In our first analysis, we divided patients into two groups, FH positive and FH negative, according to responses on the lifetime drinking history interview, and within those two groups, into late and early-onset alcoholics (LOAs and EOAs). When we found a significant FH effect, we conducted a secondary analysis where we divided patients into four groups depending on which parent was the heavy drinker

(neither, mother, father, or both). In this secondary analysis, because of smaller sample sizes, we did not divide patients into LOAs and EOAs.

6.3. Results

Participant characteristics are described in **table 6.1**. We did not find any main effects of age of onset or FH on the quantity or frequency of drinking during the six months preceding hospitalization (**table 6.2**). There were no differences in the quantity or frequency of drinking between males and females when we controlled for body size, but females had a later average age of onset than males ($F = 4.69$, $p = 0.03$).

Psychiatric history is summarized in **table 6.3**. Early-onset alcoholics had a greater number of total Axis II disorders than late onset alcoholics ($F = 18.05$, $p < 0.001$), but there were no differences in total number of mood or anxiety disorders. There was no effect of FH on psychiatric diagnoses, or on the percentage of comorbid drug abusers. Early-onset alcoholics were significantly more likely than late-onset alcoholics to have abused drugs other than alcohol ($F = 29.08$, $p < 0.0001$).

Intracranial volume

We found a significant difference in ICV among healthy controls, FH positive, and FH negative alcohol-dependent patients (F (2, 356) = 6.52, $p = 0.0017$). Post-hoc student's t-tests demonstrated a significant difference between controls and FH positive alcoholics ($p < 0.001$), and between FH positive and FH negative alcoholics ($p < 0.001$), but not between controls and FH negative alcoholics (**fig. 6.1**). A Least Squares Fit model showed that sex, height, and FH, as well as the interaction between FH and age of onset, independently accounted for significant proportions of the variance in ICV (**table 6.4**). We found a significant difference in ICV as a

function of family history in both male and female alcoholics (**fig. 6.2**). We did not find significant differences between the ICVs of EOAs and LOAs. There was a significant interaction effect of FH X age of onset (F (2,356) = 5.209. p = 0.023). In a post-hoc simple effect tests, the ICVs of FH negative LOAs were significantly greater than the ICVs of FH positive LOAs ($p <$ 0.0001). We did not find a significant difference in the ICV of FH positive compared to FH negative EOAs. Furthermore, we did not find a significant difference in ICV between EOAs and LOAs with a positive FH, but within FH negative subjects, LOAs had significantly larger ICVs than EOAs (p = 0.02) (**fig 6.3**).

In the second analysis, we divided alcoholic patients into four groups depending on which parent was a heavy drinker, again controlling for height, sex, and age. This model demonstrated a significant difference in ICV among the four groups (F (3, 242) = 4.521, p = 0.004). Pairwise post-hoc t-tests found that alcoholics with no FH had significantly larger ICVs than those with a heavy drinking father (p = 0.0133), a heavy drinking mother (p = 0.0104), and two heavy drinking parents (p = 0.0037). Moreover, FH negative males had larger ICVs than males with a heavy drinking mother or father ($p < 0.05$). In contrast, among females, FH negative patients had larger ICVs than those with a heavy drinking mother or both heavy drinking parents ($p < 0.05$), but there was no difference between female patients with a heavy drinking father and those who were FH negative (**fig 6.4**).

Brain Shrinkage

We found no main effects of FH or of age of onset of alcoholism on brain shrinkage (the brain volume/ICV ratio), and no interaction between the two measures (**table 6.5**). Predictors of brain shrinkage included age (F = 57.67, $p < 0.0001$), sex (F = 15.11, p = 0.0001), and years of heavy drinking (F = 5.02, p = 0.02). Age of onset of alcoholism did not significantly correlate with brain shrinkage in either males or females. Female alcoholics experienced significantly lower ratios of brain volume to ICV, indicating greater shrinkage, than males. There were no

significant interactions between sex and either family history or age of onset. When we examined selective shrinkage of gray and white matter volumes we found similar results, but both FH positive and FH negative alcoholics had greater brain shrinkage than healthy controls (F (1, 356) = 69.75, $p < 0.0001$).

IQ**.** Total IQ scores were predicted significantly and independently by ICV, level of education, and by FH, but not by sex, age, or age of onset (see **table 5.6**). When examined separately block design and vocabulary scores both were predicted by age. However, vocabulary significantly increased with age while block design score decreased. In addition to age, vocabulary score was also predicted by ICV, education and FH. In contrast, block design score was not predicted by ICV but was predicted by education and FH.

There was a significant interaction between the age of onset and parental drinking in both performance (block design) IQ and total IQ. For total IQ, posthoc student's t-tests indicated that FH positive LOAs had significantly lower scores than FH negative LOAs, but there was no significant difference between any of these measures in EOAs as a function of family history (**fig 6.5**). In addition, FH negative LOAs scored significantly higher than FH positive EOAs. In block design score, the same pattern emerged, with FH positive LOAs scoring significantly lower than FH negative LOAs, but no difference in the scores of EOAs as a function of FH.

Analyses conducted with patients divided into four groups again demonstrated that total IQ scores differed significantly as a function of FH (F (3,203) = 5.11, $p = 0.002$) (**fig 6.6**). Post-hoc student's t-tests indicated that FH negative patients had significantly higher scores than the FH positive patients. FH negative patients had higher block design scores, but the difference did not reach significance. In vocabulary scores, there was a significant difference as a function of FH (F (3, 203) = 4.48, $p = 0.005$), and student's t-tests indicated that FH negative patients had higher scores than FH positive patients ($p = 0.014$).

6.4. Discussion

The main finding of this paper is that adult alcoholics with a positive FH of heavy drinking have significantly smaller ICVs than alcoholics from non-alcoholic or heavy drinking families when we controlled for age, sex, and height. Brain shrinkage as measured by the ratio of brain volumes to ICV was not affected by FH. Only maximal brain and skull growth as measured by ICV was affected by FH. FH did not correlate with drinking behavior of the alcoholics themselves. Although drinking patterns may have varied throughout the lifetimes of the patients, there were no significant differences in the frequency of drinking, the quantity of drinking, total years of heavy drinking, or the age of onset of heavy drinking between the patients with a positive FH and those without. This suggests that differences in ICV between FH positive and negative alcoholics are not the result of different drinking patterns. Also, since the mean age of onset of heavy drinking, even for the EOAs, was more than 2 SDs greater than the age at which ICV growth typically ends, it is unlikely that heavy drinking contributed to differences in ICV. Less skull growth may have functional consequences in that there is a correlation between IQ and brain size (Andreasen et al 1993; De Bellis et al 1999). We found that FH positive patients had significantly lower IQ scores than patients with no parental drinking and that ICV weakly, but significantly, predicted both total IQ and vocabulary score.

The relationship between ICV and intelligence should be interpreted cautiously. Although ICV is highly heritable with an h^2 (the proportion of phenotypic variation that can be attributed to genetic causes) of about 0.9 (Baare et al 2001) ICV may be influenced by environmental factors as well. In fact, recent studies of the heritability of IQ have found that h^2 is highest when environment is optimal but is considerably lower when estimated in populations enjoying less than ideal environments (Turkheimer et al 2003). Since ICV predicts IQ it may show a similar pattern. In addition, it seems likely that alcoholics, in general, are raised in less

than optimal environments. Thus, an h^2 of 0.9 for ICV may be an overestimate in alcoholic populations. However, the mechanisms by which environment affects ICV are uncertain.

Many studies have found that living in an enriched environment positively influences central nervous system growth and development (van Praag et al 2000), while other studies have described the effects of stress on brain growth, which indicate that increased cortisol and catecholamine concentrations can modulate neuronal migration, differentiation, and synaptic proliferation in the developing brain (Lauder 1988; Sapolsky 1990; Sapolsky et al 1986; Todd 1992). In both human (Sapolsky 1996; Sapolsky et al 1986), and non-human primates (Uno et al 1989) elevated levels of stress hormones such as catecholamines and cortisol can affect brain growth by accelerating loss of neurons (Swaab et al 2005) or by delaying myelination (Dunlop 1997). COAs may experience this stress during a particularly crucial developmental stage. Between the ages of 6 months and 3 years, myelination increases dramatically and continues to increase into the third decade of life, and grey matter and limbic structures increase in volume throughout this time (Sowell et al 1999) . Therefore, the stress of growing up in an alcoholic home may affect brain growth and development, and correlate with increased risk for alcoholism during adulthood. DeBellis et al (1999) found that in maltreated children with post-traumatic stress disorder, cortisol and catecholamine concentrations correlated with the duration of maltreatment. In a subsequent study, they also found that decreased ICV was associated with the duration of maltreatment, and they propose that traumatic childhood experiences may adversely influence brain development. This is consistent with the measured heritability of ICV being lower in a more adverse environment. Although in the current study, it is not known whether children of heavy drinkers have experienced abuse or neglect, it is likely that they grew up in a more stressful environment than children of non-drinking parents. Most likely genetics and environment both contribute to the smaller ICV observed in FH positive alcoholics.

A surprising finding in the study was that the brain volumes of LOAs showed a greater effect of parental alcohol use than those of EOAs. This is, in large part, due to the FH negative

EOAs having significantly smaller ICVs than the FH negative LOAs. This difference in ICV among FH negative alcoholic groups may be related greater severity of alcoholism among the EOAs. In a clinical setting, EOAs often have more psychopathology and poorer global functioning regardless of whether their parent is a heavy drinker (von Knorring et al 1987). The EOAs in our sample had significantly higher rates of comorbid drug abuse and dependence than the LOAs as well as a considerably higher incidence of Axis II personality disorders. In a previous study with a subset of patients of the current study, EOAs scored higher on measures of impulsivity and aggression (Bjork et al 2004a). This more pathological, higher severity group may not manifest the effects of family history as clearly as other factors underpinning severe psychiatric comorbidity. Consistent with this explanation we did not find significant differences in ICV between FH positive and negative EOAs.

LOAs, in contrast, tend to have higher scores in global functioning, and alcoholism often manifests in the absence of other disorders. They have few if any social complications, few legal difficulties, and rarely act out violently while intoxicated (von Knorring et al 1987). Perhaps the differences between LOAs with and without parental heavy drinking are magnified because of the lack of other confounding factors in this "cleaner" population. Further studies are required to more thoroughly understand this effect. However, our results challenge the assumption that the genetic contribution to alcoholism necessarily manifests early in life. These data indicate that both genetics and early life environment may have profound implications that may not surface until adulthood.

We also found that among women, maternal drinking appeared to influence ICV more than paternal drinking. This makes sense, as the mother was probably the principal caretaker of the child, and more likely influenced the child's nutrition, social surroundings, and intellectual environment than the father. In addition, we have no way of assessing whether the heavy drinking mothers drank while pregnant. Although none of our participants were diagnosed with FAS, patients may have had subtle fetal alcohol effects. We did not find differences between the

effects of maternal and paternal drinking on ICV in the males in our study, suggesting that at least among males fetal alcohol effects cannot explain the smaller ICV among FH positive alcoholics. We also report larger effect size for FH on ICV among the women in our sample compared to the men, perhaps due to the more selective effect of maternal drinking on females. This suggests that women may be particularly vulnerable to either prenatal alcohol effects or postnatal environmental effects.

We found no difference in brain shrinkage between EOAs and LOAs when we controlled for age, sex, and years of heavy drinking. Brain shrinkage is independently correlated with the duration of heavy drinking after controlling for age (Bjork et al 2004a), but it appears that the time at which the drinking is initiated does not affect this process. The brains of LOAs appear to be just as susceptible to atrophy as those of younger alcoholics, which provides additional evidence that ICV reflects pre-morbid brain growth that is not sensitive to individual differences in the patient's drinking behavior. Even within the alcoholics who began heavy drinking before the age of 21 (n = 85), age of onset of alcoholism did not significantly predict brain shrinkage in either males or females. We also found that as in previous work (Hommer 2001), females are more susceptible to alcohol-induced brain shrinkage at similar alcoholism severity.

We did not find a main affect of FH on brain shrinkage, indicating that brain shrinkage occurs as a result of heavy drinking regardless of FH status. This finding contrasts with a previous study by Cardenas et al (2005), which reported that a positive FH of alcoholism was protective against brain shrinkage. However, although the Cardenas paper also looked at the effects of FH of alcoholism on brain atrophy, their methods and study population differed considerably from ours. Their primary measure of brain shrinkage was % CSF, whereas ours was a ratio of brain volume to ICV. They measured the four lobes of the brain, whereas we measured total gray matter and total white matter. In addition, their population was non-treatment seeking and they drank considerably less than our population. Therefore, their findings that family history may be protective may only be valid to a certain alcoholism severity.

Estimated IQ.

Consistent with Gabrielli and Mednick (1983), we found a significant effect of FH on estimated IQ after controlling for sex, age, and education level. Gabrielli and Mednick demonstrated that children at high risk for alcoholism had lowered verbal ability, suggesting that the lower IQs observed in alcoholics may exist before the onset of alcoholism. Since ICV is set before the onset of alcoholism, and does not change as a function of age, this is consistent with our finding that that a lower vocabulary score is associated with smaller ICV (although, it should be noted, ICV is a fairly weak predictor of IQ when education, FH and age of onset are taken into account). Block score results were not as strongly correlated with either education or FH as vocabulary results. Several studies have examined the effect of parental neglect on IQ. Cognitive, language, and intellectual impairments are frequently observed in abused and neglected children (Augoustinos 1987; Kolko 1992), and the effects may reach adulthood. In a study of adult survivors of child abuse, Perez and Widom (1994) reported lower IQ and decreased reading ability in the abused group compared to controls.

Interestingly, we found that a positive FH affected the IQ scores of the LOAs, but not of the EOAs. Again, this may be explained by the greater psychopathology of the EOAs mitigating the effect of FH on IQ through a ceiling effect. FH negative LOAs had significantly higher IQ scores than FH negative EOAs, but in the FH positive patients, both EOAs and LOAs had similar low IQ scores.

Finally, we found that EOAs had significantly higher numbers of axis II disorders than LOAs, which has been shown in many clinical samples of alcoholics (Hallman et al 1996; von Knorring et al 1987). There was no main effect of FH, suggesting that parental heavy drinking does not influence the psychiatric diagnoses of adult alcoholics.

A limitation of this study was the reliance on patients' reports of parental heavy drinking as well as use of an in-house interview instrument which did allow for a formal diagnosis of alcohol abuse or dependence. However, by the time of the interview, patients had undergone

weeks of educational alcoholism therapy sessions which directly and indirectly clarify what constitutes problematic levels of drinking. On the other hand, our classification of FH positive patients as having a "heavy drinking" parent underscores the strength of the relationship between parental drinking and ICV. Even if the heavy drinking parents would not have been diagnosed with alcohol dependence, patients raised by parents with a general pattern of heavy drinking are still affected.

An additional limitation of this study is the absence of data collected about aspects of parental lifestyles other than drinking that may have contributed to smaller ICVs of offspring, such as comorbid drug abuse or socio-economic status. We were also unable to assess maternal drinking during pregnancy. We also cannot say how well FH, ICV and IQ will predict the development of alcoholism. These are risk factors, but as with any risk factor, they do not determine that a person will develop the condition, but rather increase the likelihood that they will. To answer the question of how selective risk factors predict alcoholism we would need to select FH positive subjects on the basis of low IQ or small ICV and see if they have a higher rate of alcoholism.

Future research could more precisely study how the amount of parental drinking affects brain volumes of COAs before they are old enough to develop alcoholism themselves. Subsequent studies could also examine brain volumes of healthy controls with alcohol-dependent parents, in order to determine if smaller ICV is a more specific risk factor for the development of alcoholism than FH. Finally, more in-depth psychosocial interviewing could more directly assess parental factors on both structural development and behavioral consequences in COAs.

Table 6.1. Demographic characteristics of study groups.

Variable			Late Onset alcoholics (n = 102)	Early Onset alcoholics (n = 129)	Controls (n = 114)
Age					
	Mean (SD)		43.90 (8.48)	36.98 (8.65)	34.63 (10.13)
	Range		28-67	20-64	20-63
Sex					
	Male		51	104	54
	Female		51	25	60
Education					
	Mean Years		14.92 (2.62)	13.79 (2.55)	16.92 (2.72)
Ethnicity					
Caucasian			83	105	83
Black			17	18	16
Hispanic			1	3	7
Asian			1	0	6
Other			0	3	2
Family History					
	FH -		38	47	114
	FH +		64	82	0
		Mother	12	12	
		Father	35	42	
		Both	17	28	

Table 6.2. Drinking behavior and co-morbid drug abuse of study groups.

	Early-Onset		Late-Onset	
	FH -	FH +	FH -	FH +
Mean Age of Onset (SD)	18.98 (2.49)	19.32 (2.87)	33.76 (7.45)	33.03 (7.47)
Range	14-24	13-24	25-55	25-59
Mean Quantity Consumed (SD)	14.87 (6.87)	13.46 (7.3)	11.23 (5.87)	11.69 (7.07)
Mean Drinking Frequency (SD)	26.08 (6.94)	21.9 (10.98)	24.34 (8.85)	25.37 (8.13)
Mean Years of Heavy Drinking (SD)	15.07 (7.94)	13.04 (7.19)	8.65 (6.59)	8.79 (6.81)
% Comorbid Drug Abusers*	72 %	71 %	39 %	48 %

*A significantly greater percentage of early-onset than late-onset alcoholic patients were characterized as comorbid drug abusers ($p < 0.05$). There were no significant differences among groups in quantity consumed, drinking frequency, or years of heavy drinking.

Table 6.3. Psychiatric diagnoses of study groups.

Total # Lifetime Disorders		Early-Onset		Late-Onset	
Mood		FH -	FH +	FH -	FH +
	0	13%	27%	14%	14%
	1	53%	31%	32%	46%
	2	28%	35%	40%	33%
	3	4%	6%	13%	6%
Mean (SD)		1.30 (2.58)	1.27 (3.02)	1.54 (2.89)	1.49 (1.92)
Anxiety					
	0	55%	53%	67%	55%
	1	30%	32%	19%	35%
	2	13%	12%	8%	7%
	3	2%	2%	5%	2%
Mean (SD)		0.62 (0.83)	0.64 (1.12)	0.51 (0.90)	0.62 (1.17)
Axis II					
	0	10.5%	14.8%	40%	21.8%
	1	19.1%	13.6%	10.8%	21.8%
	2	8.5%	11.1%	8%	18.2%
	3	12.8%	9.9%	16.2%	9.1%
	4	10.6%	7.4%	5.4%	10.9%
	> 4	38.5%	43.2%	19.6%	18.2%
Mean (SD)		3.62 (0.80)	4.0 (0.80)	2.41 (0.87)	2.25 (0.85)

Table 6.4. Factors affecting brain volume measures in alcoholic patients.

	r^2 (complete model)	Sex	Height	Age	Family History	Age of Onset	FH x Age of Onset
ICV	0.33	$F = 20.28$ $p < 0.0001$	$F = 11.88$ $p = 0.001$	NS	$F = 13.23$ $p < 0.0001$	NS	$F = 5.209$ $p = 0.023$
Brain / ICV	0.30	$F = 5.69$ $p = 0.0179$	NS	$F = 61.337$ $p < 0.001$	NS	NS	NS
Gray/ ICV	0.39	NS	NS	$F = 105.75$ $p < 0.001$	NS	NS	NS
White/ ICV	0.19	$F = 9.92$ $p = 0.002$	NS	$F = 13.43$ $p < 0.001$	NS	NS	NS

The value of r^2 equals the amount of variance explained by all of the factors (sex, height, age, family history, age of onset, and the interaction between family history and age of onset) included in the model. NS = non-significant.

Table 6.5. ICV and brain shrinkage values in healthy controls and alcoholic patients.

	Controls		FH -		FH +	
	Mean	SD	Mean	SD	Mean	SD
ICV	1323.89	129.65	1349.89	113.14	1296.48*	121.28
Brain/ICV	0.824*	0.028	0.790	0.032	0.796	0.034
Gray/ICV	0.441*	0.021	0.419	0.020	0.423	0.021
White/ICV	0.382*	0.035	0.371	0.016	0.372	0.017

An asterisk indicates a significant difference ($p < 0.05$) from the other two groups.

Table 6.6. Factors affecting estimated IQ in alcoholic patients.

	r^2 (complete model)	ICV	Education	Age	Family History	Age of Onset	Family History x Age of Onset
Total IQ (Estimated)	0.27	$F = 4.389$ $p = 0.038$	$F = 22.587$ $p = < 0.0001$	NS	$F = 7.62$ $p = 0.007$	NS	$F = 4.85$ $p = 0.029$
Vocabulary IQ	0.35	$F = 4.025$ $p = 0.042$	$F = 27.158$ $p < 0.0001$	$F = 4.271$ $p = 0.041$	$F = 5.857$ $p = 0.017$	NS	NS
Block IQ	0.19	NS	$F = 6.344$ $p = 0.013$	$F = 14.839$ $p = 0.0002$	$F = 3.993$ $p = 0.049$	NS	$F = 5.646$ $p = 0.019$

The value of r^2 equals the amount of variance explained by all of the factors (ICV, education, age, family history, age of onset, and the interaction between family history and age of onset) included in the model. NS = non-significant.

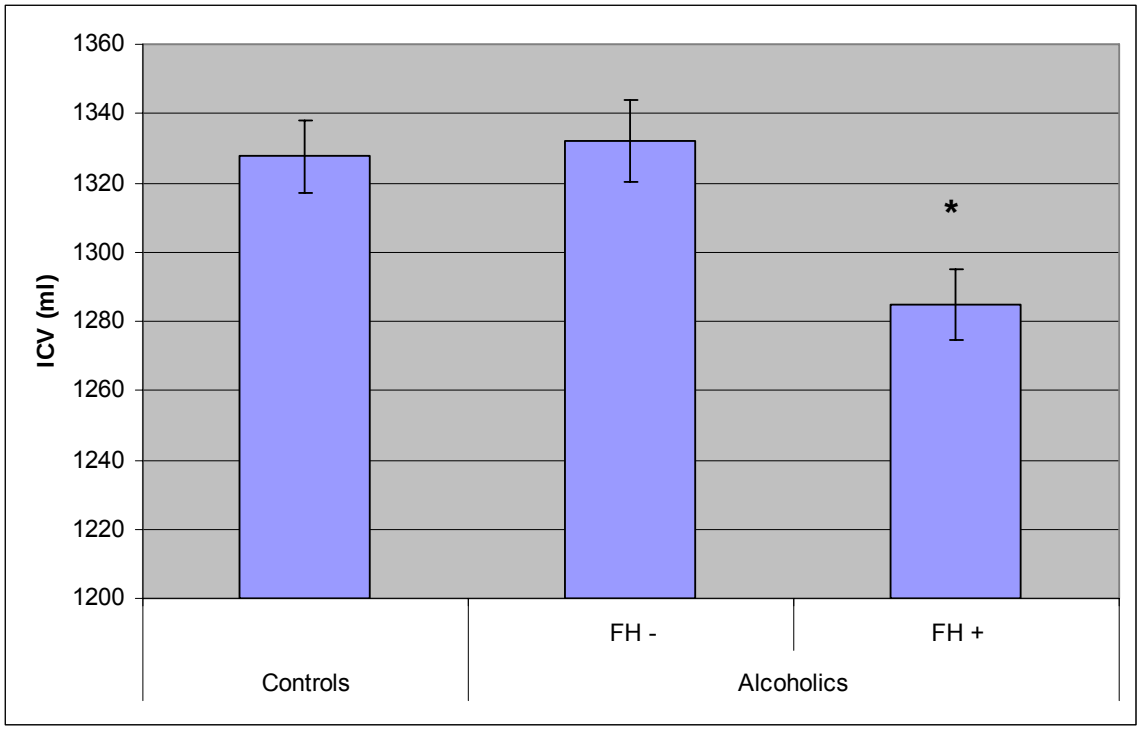


Fig 6.1. Adjusted means of ICV in controls and alcoholic patients. We found a significant difference in ICV among healthy controls, FH positive, and FH negative alcoholic patients (F (2, 356) = 6.52, p = 0.0017). Post-hoc student's t-tests demonstrated a significant difference between controls and FH positive alcoholic patients, and FH negative and FH positive patients, but not between controls and FH negative alcoholics. Error bars indicate SEM.

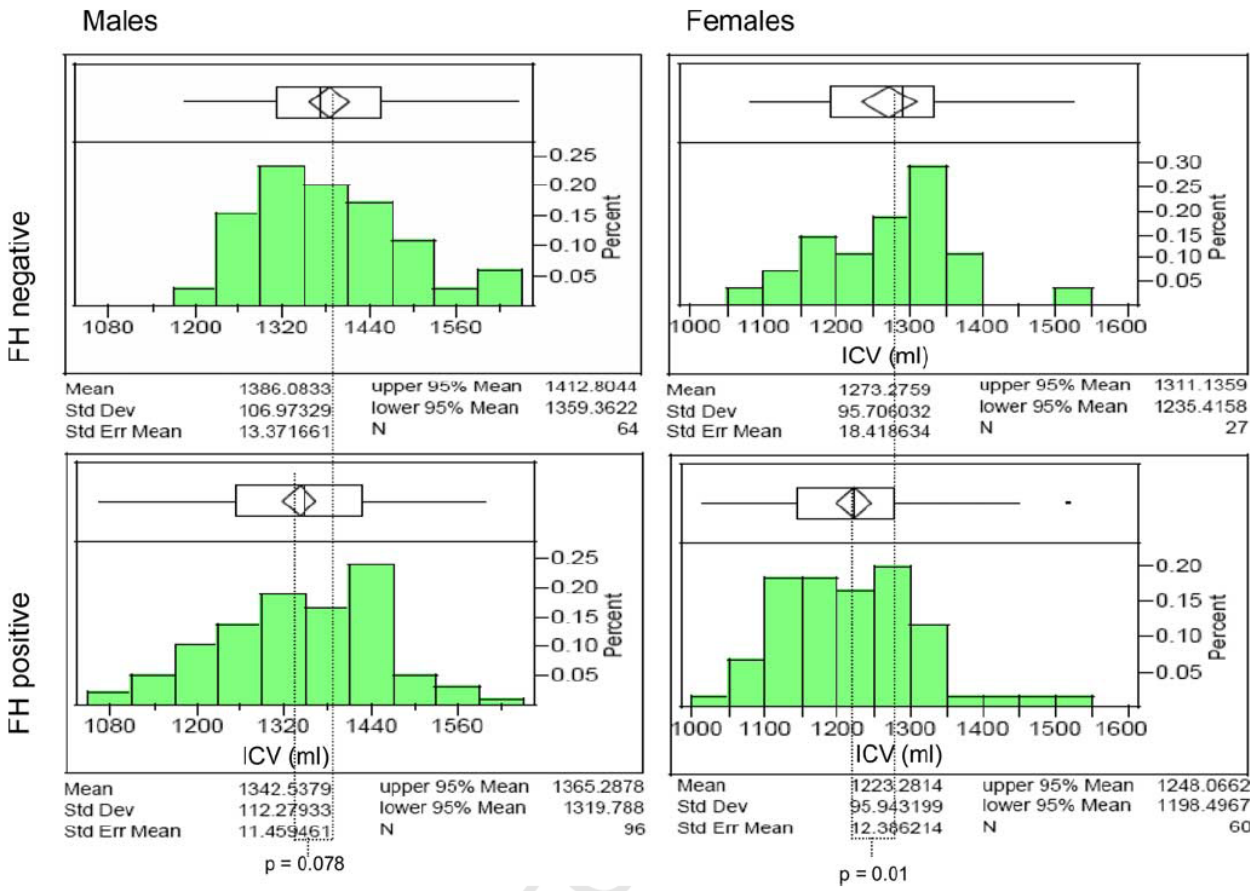


Fig 6.2. ICVs of male and female alcoholic patients. The rectangular box above each distribution shows the middle half of the data. The solid line within the box represents the median value. The whiskers that extend out from the box show the tails of the distribution, and any points outside of the whiskers are possible outliers. The solid line connecting the FH + and FH - panels represents the mean value for each cell.

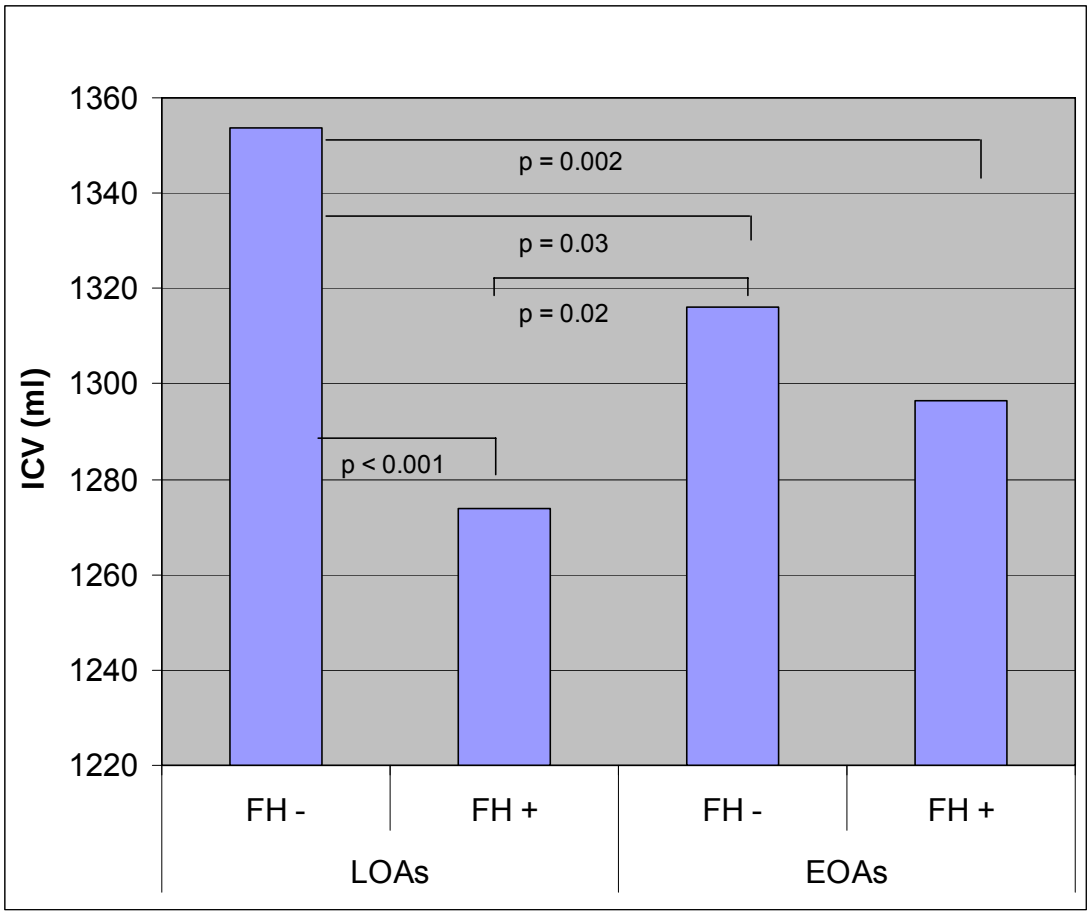


Fig 6.3. Adjusted means of intracranial volume in LOAs and EOAs. We found a significant effect of FH (F (1, 224) = 13.23, $p < 0.0001$) and an interaction between FH and age of onset (F (1, 244) = 5.209, $p = 0.023$). Bars indicate significant results of a student's t-test among the four groups.

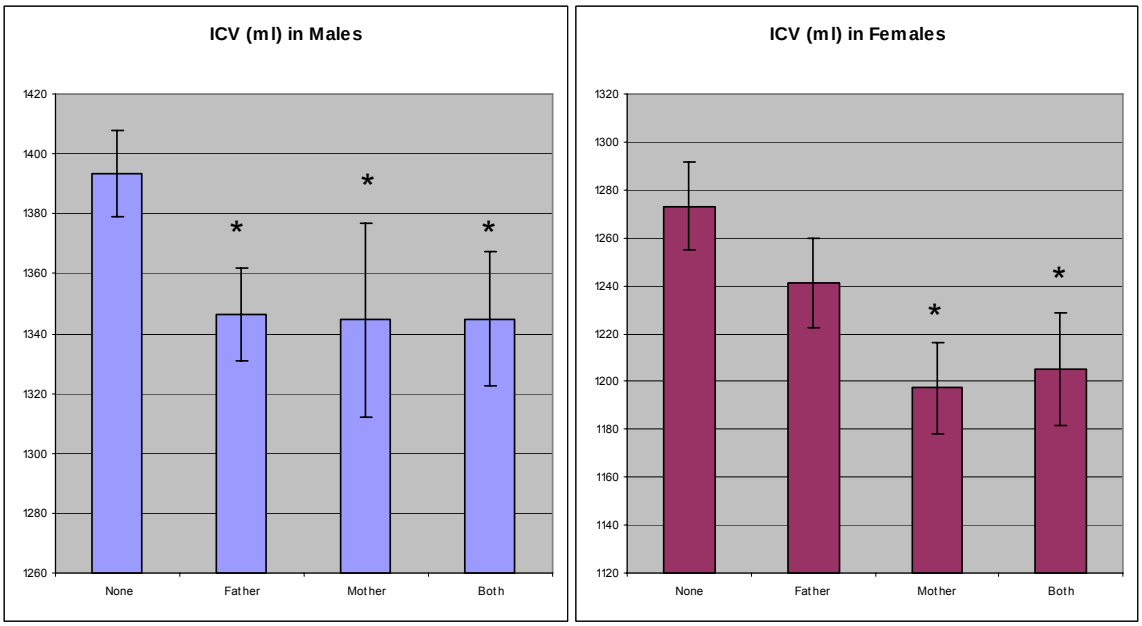


Fig 6.4. Adjusted means of ICV in alcoholic patients by sex. We generated adjusted means for ICV after co-varying for sex and height. The ICVs of FH negative patients were significantly larger than those of FH positive patients. An asterisk indicates that the mean is significantly different from "none." Error bars indicate SEM.

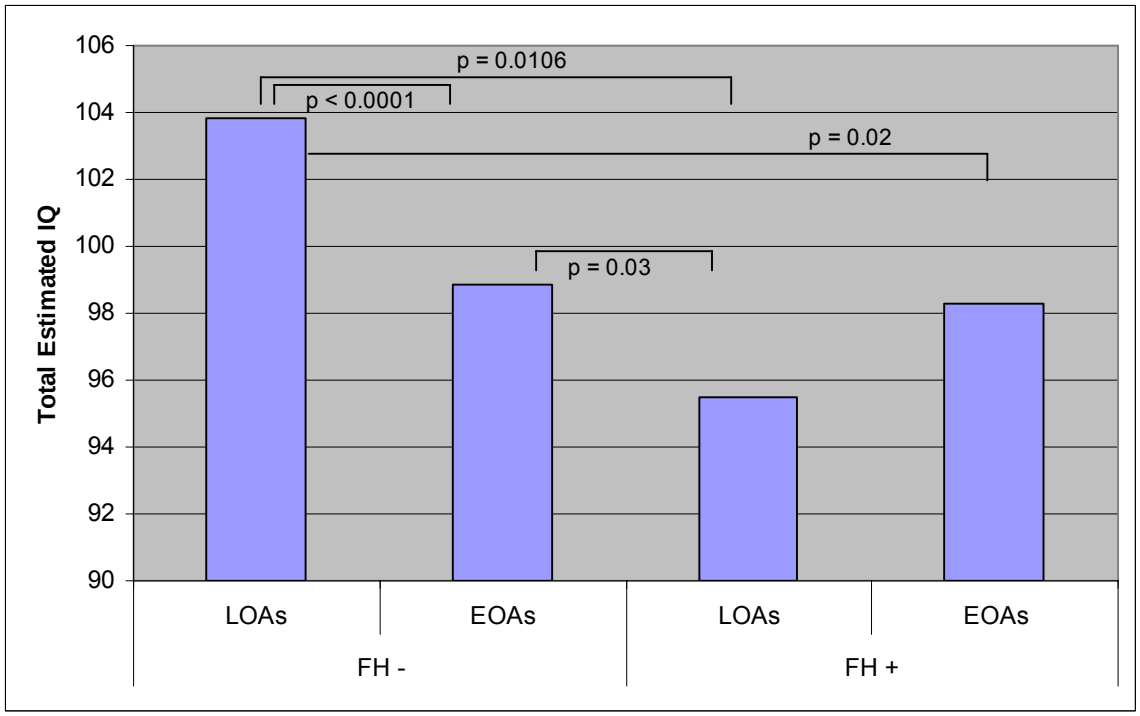


Fig. 6.5. IQ Scores of EOAs and LOAs by family history. We found a significant effect of FH (F (1, 224) = 11.202, p = 0.001) and an interaction between FH and age of onset (F (1, 244) = 8.85, p = 0.003). Bars indicate significant results of a student's t-test among the four groups.

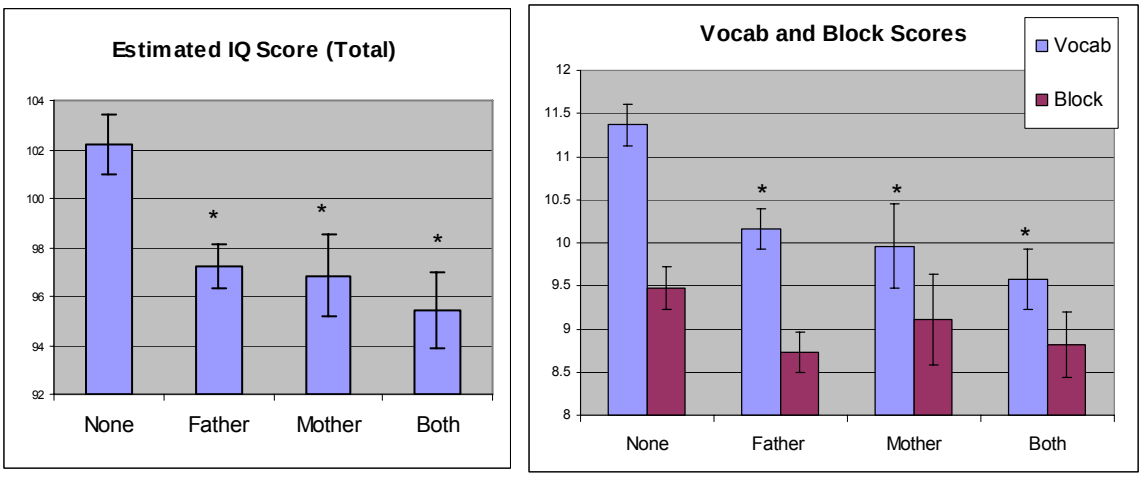


Fig. 6.6. Estimated IQ Scores of alcoholic patients. (Left) FH negative patients had significantly higher scores than FH positive patients. (Right) FH negative patients had significantly higher vocabulary scores than FH positive patients. An asterisk indicates that the mean is significantly different from "none." Error bars indicate SEM.

Chapter 7:

Summary and Conclusions

Seeking reward and avoiding punishment are two of the most basic instincts of any living animal. These instincts cause us to seek food, shelter, companionship, and other pleasurable outcomes, as well as to avoid injury and harm. The data from this thesis suggests that addicted patients have a compromised neural response to appetitive and aversive stimuli, which may partially explain the continued use of drugs despite negative or punishing consequences. This report, however, is only a beginning. Emotional instability, reflected in neural circuitry involved in pleasure or fear, must be further studied in order to understand how we can better guide future treatments for alcoholism and other addictive disorders. In addition, studying how the brain responds to centrally acting compounds, especially alcohol due to its ability to modulate mood states, will increase our understanding of how the brain processes incoming affective information.

7.1. Overview

The objective of this thesis was to better understand the how acute alcohol administration and prolonged heavy alcohol consumption are related to the neural correlates of emotion. Although it is well-known that alcoholic patients experience difficulty regulating mood states, only recently have researchers begun to study emotional processing in alcoholic patients. Research on the neural correlates of emotion under the influence of alcohol is even harder to find; to our knowledge, we are the first group to use brain imaging to study emotional processing under intoxicated conditions. It is our hope that the work presented in this report will encourage researchers in the field of addiction to more seriously consider brain differences in the processing of emotion as a predictor (or an effect) of substance abuse.

Our work largely supports the theory of George Koob, who states that post-dependent individuals activate the anti-reward system, causing a constant aversive, stress-like mood state (Koob 1992). In Chapter 2, we show an increased reactivity to negative images in alcoholic patients relative to controls in the parahippocampal gyrus, lingual gyrus, and temporal regions associated with the processing of emotion. We also found that the alcoholic patients showed less activity than controls to positive images, which supports Nora Volkow's hypothesis that the intense high caused by drugs of abuse desensitize substance abusers to natural rewards (Volkow et al 2003). Chapter 2 also showed us that these differences in emotional reactivity can be modulated through conditioned alcohol cues. When images were presented with alcohol cues, alcoholic patients became more reactive to positive and less reactive to negative images, suggesting that cues that have become associated with alcohol may be used to reduce negative and enhance positive affect. This study also suggested that BOLD activation to emotional images in specific brain regions can be used as a biomarker in alcoholism. We exploited this idea in Chapter 3, where we repeated the same experiment as part of a phase II clinical trial. We found that patients being treated with a novel anxiolytic drug, LY686017, has less activation to negative

images and greater activation to positive images than patients on a placebo treatment. This study accomplished two goals: first, it showed that emotional processing could be modulated though a drug targeting the stress-related neurotransmitter substance P, and second, it showed that pharmacological fMRI could be a useful method to study alcoholism.

In Chapter 4, we designed a decision-making task to engage the frontal brain regions, which are implicated in emotional evaluation and are also particularly vulnerable to alcohol's neurotoxic effects. We found that when alcoholic patients were required to make decisions about emotional images, they exhibit greater activation than controls in several frontal and limbic brain regions. This greater activation occurred regardless of the emotional valence of the image, suggesting that the evaluation of any highly-emotional image requires alcoholics to recruit brain regions that controls do not need to use in order to complete the task. This recruitment of frontal systems in a simple, two-choice task suggests inefficiency in alcoholics' cognitive functioning and may explain their inability to regulate mood states in more complex situations.

The results of Chapters 2, 3, and 4 provided a better understanding of the emotional deficits experienced in alcoholic patients. Might alcoholics use alcohol to modulate these emotional deficits? In Chapter 5, we examined how alcohol itself affected the neural correlates of emotion in social drinkers. We found that intravenously administered alcohol activated pleasure-related centers of the brain, while attenuating the neural response to fearful faces in emotional-visual brain regions. This suggested that alcohol could be used to modulate mood states, especially in social settings.

Finally, Chapter 6 examined brain growth and shrinkage in an alcoholic population. By measuring intracranial volume using MRI, we found that alcoholic patients with a family history of heavy drinking had smaller maximal brain growth than alcohol patients without a family history of drinking. Since intracranial volume peaks before most alcoholic patients begin drinking heavily, this difference in not caused by alcohol consumption. This suggests that reduced brain size may be a genetic or environmental risk factor in the development of alcoholism.

7.2. Implications

These data suggest that alcohol dependence becomes a reinforcing cycle (**fig 7.1**). In alcoholic patients, a constant state of negative affect / lack of positive affect leads to increased alcohol consumption, which may cause a temporary alleviation of this mood dysregulation. This reinforcement leads to the maintenance of alcoholism, which itself which leads to increasingly dysregulated mood states, all of which can be influenced by genetic/environmental factors. Any treatment for alcoholism must necessarily break that cycle, and the three currently available pharmacological treatments for alcoholism attempt to do just that. Disulfurmam (Antabuse), the first drug approved for alcoholism, works by making alcohol consumption so aversive that alcoholic patients no longer find it rewarding, and instead the alcohol becomes a punishing stimulus. This interrupts the link in the model from consumption to reinforcement. Naltrexone, the second of the three pharmacological treatments for alcoholism on the market today, is an opiate antagonist that is thought to module the dopaminergic mesocorticolimbic pathway, which is also thought to decrease the rewarding effects of alcohol (Anton et al 2008). Acamprosate, the third currently available treatment, works possibly by interacting with glutamatergic and GABAergic systems in the nucleus accumbens (Berton et al 1998), which also may decrease alcohol's reinforcing effects. These drugs, however, are only marginally effective in most patients. Antabuse is ineffective due to poor compliance, and Naltrexone only appears to be effective in a subset of patients (Anton et al 2008). The efficacy of Acamprosate is also questionable. The COMBINE study, the largest study in the US to investigate the efficacy of Acamprosate, did not show that Acamprosate was effect either alone or in combination with Naltrexone for preventing relapse (Anton et al 2006). In another double-blind, placebo controlled trial of Acamprosate, researchers found no significant difference between patients treated with Acamprosate and placebo in percentage of abstinent days, but did find a positive effect *post-hoc*

in a subset of patients who were motivated to have a treatment goal of complete abstinence (Mason et al 2006).

The incomplete effectiveness of current treatment suggests that perhaps treating emotional disregulation is as important as reducing the reinforcing / rewarding properties of alcohol. Self-report evidence supports this notion. Craving, or the obsessive drive to seek out and find the drug, is often associated with negative affect, and negative affect is one of the most common antecedents of relapse (Brownell et al. 1986, McKay et al. 1999). In fact, urges to drink induced by alcohol-associated cues are closely correlated with levels of self-reported anxiety (Monti et al. 1993). Our NK1 antagonism study (Chapter 3) attempts to treat negative affect before it leads to alcohol consumption. While it is too soon to predict whether LY686017 will become an effective treatment for alcoholism, we hope that emphasis is placed on treating the mood dysregulation as well as on decreasing the rewarding properties of alcohol when developing new alcoholism therapies.

7.3. Future Directions

Researchers are only beginning to understanding how alcohol affects the brain. Both long-term effects of heavy alcohol consumption and short-term affects of alcohol administration must be further studied to complete our understanding of this substance. As in any human experiment, different populations need to be studied in order to generalize our results. We have shown that alcoholic patients show an increased brain response to negative images, but our studies have included an alcoholic population that presented significant comorbid drug abuse and comorbid psychiatric diagnoses. Future studies can examine groups such as alcoholics with and without mood disorders, and with and without comorbid drug abuse, in order to determine how these factors contribute to our findings. Future studies can also examine these effects in recovered alcoholics, in order to determine if the differential response to emotional images

persists even after long periods of sobriety. Another interesting study population is children of alcoholics (COAs) who may be a higher risk than control children for developing alcoholism.

Our alcohol infusion study (Chapter 5) offers evidence of how acute alcohol affects the brain, but future experiments should study this in alcoholic patients, in order to examine if the brains of addicted patients respond to alcohol differently than those of social drinkers. Although there are many ethical issues to consider when designing this type of study, we feel that this data will be scientifically relevant in the design of better treatments for alcoholism. Currently available pharmacological treatments attempt to alter the mesocorticolimbic reward pathway, but we know little about how this pathway functions in alcoholic patients.

Future studies can also examine the acute response to alcohol in COAs. Preliminary studies in our laboratory have shown that COAs have less striatal tissue than control children when adjusted for intracranial volume, and this may reflect an increased (or decreased) sensitivity to alcohol. Finally, studies can use different doses of alcohol in order to determine the necessary amount of alcohol that will reliably activate the ventral striatum, and how individual differences can affect this amount. Studies can also use different time courses of exposure, or compare striatal activity in the ascending versus descending limb of the blood alcohol curve.

Finally, this thesis suggests that pharmacological fMRI can be a useful technique for addressing the effects of drugs in the brain. Traditionally, PET has been the preferred method of assessing drug effects, due to its direct measure of receptor occupancy of the drug. However, as discussed in Chapter 3, fMRI has several advantages over PET, such as non-invasiveness, potential for with-in subject designs, and superior spatial and temporal resolution. Our pharmacokinetically controlled alcohol challenge (Chapter 5) shows that fMRI can be sensitive to local effects of drugs in particular regions of the brain. This experiment also demonstrates that a jittered-interval event-related design fMRI design is sensitive to different aspects of a drug's effects on the brain depending on the task or stimuli. The LY686017 clinical trial is another example of how event-related fMRI can be used to look at the effects of drugs in the brain during

a specific, event-related task. Pharmacological fMRI has the potential to offer unique insight into the effects of centrally acting compounds on brain activity, and is useful from both a pharmaceutical perspective and a basic science perspective. Future studies can use pharmacological fMRI to yield data on the effectiveness of drug treatments, the possible short-term and long-term effects of drugs on brain activity, and the brain systems that are targeted by the drug. With this method, we can increase our understanding of how centrally acting compounds can affect the neural circuits involved in reward, motivation, fear, pleasure, and other cognitive brain circuits.

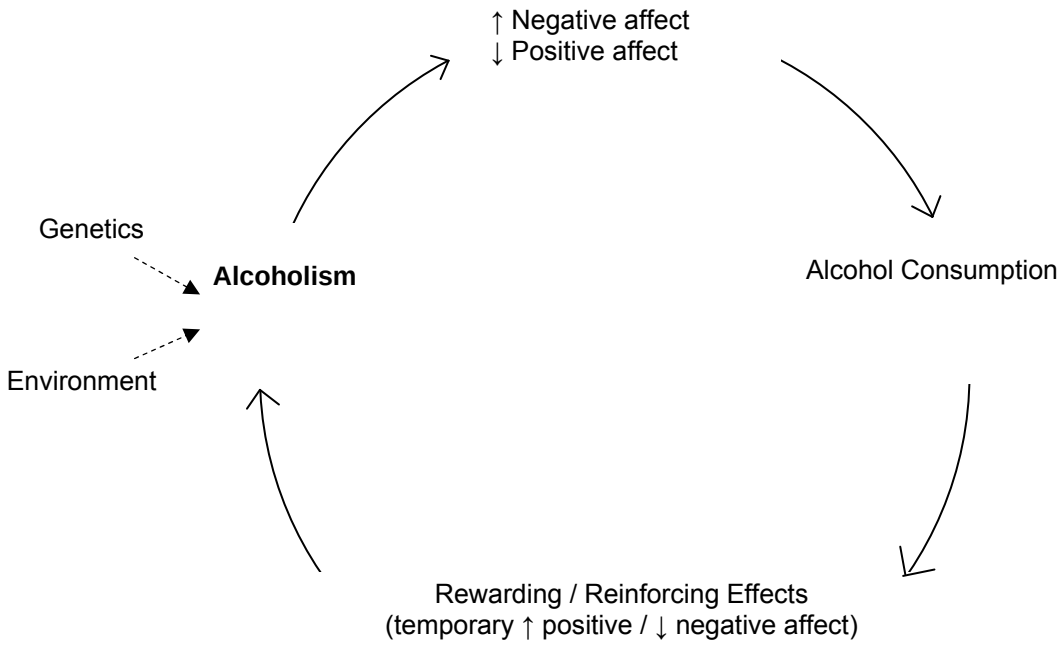


Fig 7.1. Cycle of emotional dysregulation, alcohol consumption, reinforcement, and alcoholism. According to this model, decreased positive / increased negative emotion is both a cause and an effect of alcohol. This negative emotional state is a factor in the development and the maintenance of alcoholism.

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