UNIVERSITY OF CALIFORNIA, SAN DIEGO

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Adolescent Alcohol Use and fMRI BOLD Response: A Longitudinal Study

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Clinical Psychology

by

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The Dissertation of Lindsay M. Squeglia is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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The primary analyses from this work have been accepted for publication in the *Journal of Studies on Alcohol and Drugs* as "Brain Response to Working Memory Over Three Years of Adolescence: Influence of Initiating Heavy Drinking." This dissertation author is the primary author of this material along with co-authors Drs. Susan Tapert, Carmen Pulido, Reagan Wetherill, Joanna Jacobus, and Gregory Brown.

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"Impact of Alcohol Use on Adolescent fMRI BOLD Response: A Longitudinal Study"

Aims: prospectively examine brain response to a visual working memory task in adolescents who transition into heavy drinking versus youth who remain non-users using blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI), and examine if these neural abnormalities are associated with neuropsychological functioning.

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2000	Nancy P. Mayer Scholar
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PUBLICATIONS

Peer-reviewed Data-Based Papers

1. **Squeglia, L. M.**, Spadoni, A. D., Infante, M. A., Myers, M. G., & Tapert, S. F. (2009). Initiating moderate to heavy alcohol use predicts changes in neuropsychological functioning for adolescent girls and boys. *Psychology of Addictive Behaviors*, 23 (4), 715-22. PMID: 20025379.

2. **Squeglia, L. M.**, Dager Schweinsburg, A., Pulido, C. & Tapert, S. F. (2011). Adolescent binge drinking linked to abnormal spatial working memory brain activation: differential gender effects. *Alcoholism: Clinical and Experimental Research,* 35 (10), 1831-41. PMID: 21762178.

3. Norman, A. L., Pulido, C., **Squeglia, L. M.**, Spadoni, A. D., Paulus, M. P., & Tapert, S. F. (2011). Neural activation during inhibition predicts initiation of substance use in adolescence. *Drug and Alcohol Dependence,* 119 (3), 216-23. PMID: 21782354.

4. **Squeglia, L. M.,** Sorg, S. F., Dager Schweinsburg A., Wetherill, R. R., Pulido, C. & Tapert, S. F. (2012). Binge drinking differentially affects adolescent male and female brain morphometry. *Psychopharmacology*, 220 (3), 529-39. PMID: 21952669.

5. Ray, L. A., Courtney, K. E., Bujarski, S., & **Squeglia, L. M.** (2012). Pharmacogenetics of alcoholism: A clinical neuroscience perspective. *Pharmacogenomics,* 13 (2), 129-32. PMID: 22256863.

6. **Squeglia, L. M.,** Pulido, C., Wetherill, R. R., Jacobus, J., Brown, G. G., Tapert, S. F. (in press). Brain response to working memory over three years of adolescence: Influence of initiating heavy drinking. *Journal of Studies on Alcohol and Drugs.*

Book Chapters and Review Papers

1. Amstadter, A. B., & **Squeglia, L. M.** (2007). Application of dialectical behavior therapy to disorders other than borderline personality disorder: A critical review. *Graduate Student Journal of Psychology,* 9, 16-24.

2. **Squeglia, L. M.**, Jacobus, J., & Tapert, S. F. (2009). The influence of substance use on adolescent brain development. *Clinical EEG & Neuroscience,* 40, 32-39. PMID: 19278130.

3. Bazinet, A. D., **Squeglia, L. M.**, Tapert, S. F., & Riley, E. P. (in press). The effects of drug exposure on development. In K.J. Sher (Ed.), Oxford Handbook of Substance Use Disorders. Oxford: University Press.

4. Dager Schweinsburg A., **Squeglia, L. M.,** Castro, N. & Tapert, S. F. (in press). Addiction and the Human Adolescent Brain. In: P. Miller (Ed.), Encyclopedia of Addictive Behaviors.

Manuscripts Under Review

1. Wetherill, R. R., Castro, N., **Squeglia, L. M.,** & Tapert, S. F. (submitted). Atypical frontoparietal activity during inhibitory processing in substance-naïve youth who later experience alcohol-induced blackouts.

2. Pulido, C., Cummins, K., **Squeglia L. M.,** Infante, M. A., & Norman, A. L. (submitted). Alcohol cue reactivity: Performance equivalence to a neuroimaging task.

3. Medina, K. L., Thayer, R., **Squeglia, L. M.,** McQueeny, T. & Tapert, S. F. (submitted). Recent binge drinking predicts smaller cerebellar volumes in adolescents.

PROFESSIONAL PRESENTATIONS

1. Jelsone, L., Kraft, M., Pappas, S., **Squeglia, L.**, Rorden, C., and Baylis, G. (2004, March). *Effect of temporal and spatial bias on visual extinction.* Poster presented at the Annual Cognitive Neuroscience Society conference, Charleston, SC.

2. **Squeglia, L. M.**, Amstadter, A. B., Thomas, S. E., Book, S. W., & Randall, C. L. (2007, July). *Paroxetine decreases ruminative thoughts in socially anxious alcoholics: A pilot study.* Poster presented at Research Society on Alcoholism Conference, Chicago, IL.

3. Spadoni, A. D., **Squeglia, L. M.**, Norman, A. L., Tapert, S. F. (2008, February). *Utility of baseline neurocognitive performance in predicting subsequent use in adolescence.* Poster presented at International Neuropsychology Society Conference, Waikaloa, HI.

4. **Squeglia, L. M.**, Spadoni, A. L., Gorlick, A. S., Norman, A. L., Eberson, S. C. & Tapert, S. F. (2008, June). *Effects of hangover on neurocognition in adolescents: A*

prospective study. Poster presented at the Research Society on Alcoholism, Washington, DC.

5. **Squeglia, L. M.**, Infante, M. A., Hanson, K. L., & Tapert, S. F. (2009, February). *Can neuropsychological assessment predict future drinking in adolescents?* Poster presented at the International Neuropsychological Society Conference, Atlanta, GA.

6. **Squeglia, L. M.**, Pulido, C., Spadoni, A. D., Infante, M. A. & Tapert, S. F. (2009, June). *Heavy drinking adolescents show reorganized activation patterns during visual working memory.* Poster presented at the Research Society on Alcoholism, San Diego, CA.

7. Pulido, C., **Squeglia, L. M.**, Eberson, S. C., Infante, M. A., Lentz, S., Boucquey, V., Paulus, M. P., Tapert, S.F. (2010, June). *Non-drinking adolescents with a family history of alcohol use disorders do not show alcohol cue BOLD response*. Poster presented at the Research Society on Alcoholism, San Antonio, TX.

8. **Squeglia, L. M.**, Pulido, C., Infante, M. A., Castro, M., & Tapert, S. F. (2010, June). *Heavy drinking adolescents show reorganized brain activation patterns during visual working memory: A longitudinal study*. Poster presented at the Research Society on Alcoholism, San Antonio, TX. **invited for oral presentation*

9. **Squeglia, L. M.**, Dager Schweinsburg, A., Sorg, S. F., Boucquey, V., Castro, N., Eberson, S. C., Wetherill, R. R., Pulido, C., & Tapert, S. F. (2011, June). *Does binge drinking during adolescence interfere with normal brain development?* Poster presented at the Research Society on Alcoholism, Atlanta, GA.

10. Wetherill, R. R., Bava, S. Boucquey, V., Pulido, C., **Squeglia, L. M.**, & Susan F. Tapert. (June, 2011). *Altered frontoparietal connectivity in substance-naïve youth with a family history of alcoholism*. Poster presented at the Research Society on Alcoholism, Atlanta, GA.

11. **Squeglia, L. M.,** Courtney, K. E., Bujarski, S., & Ray, L. A. (June, 2012). *Dopaminergic genetic associations with impulsive decision-making in problem drinkers.* Poster to be presented at the Research Society on Alcoholism, San Francisco, CA.

12. Jacobus, J., **Squeglia, L. M.,** Bava, S. & Tapert, S. F. (June, 2012). *Longitudinal characterization of adolescent substance use*. Oral presentation to be presented at the Research Society on Alcoholism, San Francisco, CA.

13. Wetherill, R. R., **Squeglia, L. M**., Tapert, S. F. (June, 2012). *Brain response during inhibitory processing across adolescence: Effects of heavy drinking.* Oral presentation to be presented at the Research Society on Alcoholism, San Francisco, CA.

RESEARCH EXPERIENCE

2003-2004	Attention and Perception Laboratory University of South Carolina/Health South Rehabilitation Center Columbia, SC Research Assistant Supervisor: Gordon Baylis, Ph.D.
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CLINICAL EXPERIENCE

2008-2009	Psychology Clinic at San Diego State University, Psychotherapy Trainee Supervisors: Elizabeth Klonoff, Ph.D., Director, Nader Amir, Ph.D., Linda Gallo, Ph.D.
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2010-2011	Neuropsychological Assessment Unit, VA San Diego Healthcare System, Psychology Trainee Supervisors: Dean Delis, Ph.D., Greg Brown, Ph.D., Mark Bondi, Ph.D., Vincent Filoteo, Ph.D.
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JOURNAL REVIEWER

Drug and Alcohol Dependence, ad hoc co-reviewer
Human Brain Mapping, ad hoc co-reviewer
NeuroImage, ad hoc co-reviewer
Psychology of Addictive Behaviors, ad hoc co-reviewer

PUBLIC SERVICE AND DATA DISSEMINATION

7/11 Press release on neural effects of girls' binge drinking in *WebMD*, *BBC*, *MetroFrance*, *International Business Times, United Press International, Medical News Today.*

7/11 Radio interview on neural effects of adolescent girls' binge drinking for *Wall Street Journal Radio*.

11/11 Newspaper interview for *Toronto Star* for the 2011 Atkinson Series on Women and Alcohol Use.

12/11 *Neurology Now* article on the effect of alcohol use on adolescent brain development.

12/11 Brief Report on *Betty Ford Sci-Mat website* regarding the effect of adolescent binge drinking on brain morphology.

3/12 Radio interview for syndicated Dr. Carol Show. Topic: Alcohol's Effect on Adolescent Brain Functioning.

5/12 *American Scientific Mind* interview on alcohol's effect on brain development.

PROFESSIONAL MEMBERSHIP

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ABSTRACT OF THE DISSERTATION

Adolescent Alcohol Use and fMRI BOLD Response: A Longitudinal Study

by

Lindsay M. Squeglia

Doctor of Philosophy in Clinical Psychology

University of California, San Diego, 2012
San Diego State University, 2012

Professor Susan F. Tapert, Chair

Background: Many adolescents engage in heavy alcohol use. From existing literature it is difficult to disentangle whether brain abnormalities are a consequence of heavy drinking, a preexisting risk factor for initiation of alcohol use, or both.

Methods: This study uses longitudinal functional magnetic resonance imaging (fMRI) data from 12 to 16 year-olds (*N*=40) imaged prior to the onset of drinking, then again approximately three years later after half transitioned to heavy drinking (80 total scans). Heavy drinkers and non-users were matched on baseline and follow-up developmental and risk factors. A repeated measures group x time ANOVA on *a priori* specified regions of interest was conducted to determine if youth who initiated heavy drinking evidenced a change in activation pattern, as compared to youth who remained non-drinkers. Regions showing divergent activation among initiators of heavy drinking were examined for correspondence with neuropsychological measures of VWM and attention among the heavy drinkers (*n*=20) in regression analyses.

Results: As hypothesized, significant group x time interactions were found in the right inferior parietal lobule (cluster size: 810μL, p=.005; η^2=.23) and left medial frontal gyrus (cluster size: 1431μL, p=.003; η^2=.19). For both interactions, heavy drinkers showed significantly less blood oxygen level dependent (BOLD) response contrast to high relative to low working memory loads at baseline that increased after the onset of heavy drinking, as compared to controls. Contrary to hypotheses, BOLD response contrast and its change over time were not related to follow-up neuropsychological performance.

Discussion: Adolescents who initiated heavy drinking had different brain activation compared to non-drinkers prior to the onset of drinking, suggesting brain activation patterns could be a risk factor for future substance use. Over time, adolescent heavy drinkers exhibited less efficient and mature processing of information. While brain activation did not correlate with behavioral measures, continued heavy use during this important developmental period could compromise neural networks. This investigation helps clarify the effect of alcohol use on brain functioning during adolescence, and aids in understanding whether abnormalities in VWM response among adolescent drinkers follow the initiation of alcohol involvement or predate the onset of drinking.

INTRODUCTION

Alcohol Use Increases Dramatically during Adolescence

Adolescence is a transitional period between childhood and adulthood that is marked by unique biological, psychological, and social transformations. This phase of development coincides with significant increases in alcohol consumption, with past year rates of alcohol use increasing from 29% to 65% and past year drunkenness rising from 12% to 44% between 8th and 12th grade (Johnston, O'Malley, Bachman, & Schulenberg, 2011). Heavy episodic drinking is also common among youth and particularly concerning, as 23% of 12th graders report drinking five or more drinks on one occasion during the past two weeks (Johnston et al., 2011). In addition to increased drinking and bingeing behaviors, substance-related clinical disorders begin to emerge during adolescence, with 5% of youth ages 12 to 17 meeting diagnostic criteria for an alcohol use disorder (SAMHSA, 2008).

The Brain Continues to Develop During Adolescence

Adolescence marks a critical period of neurodevelopment. The adolescent brain undergoes significant anatomical, functional, neurochemical, and hormonal changes to create a more refined, efficient central nervous system (Crews, He, & Hodge, 2007; Durston et al., 2001; Gogtay et al., 2004; Paus et al., 1999; Schweinsburg, Nagel, & Tapert, 2005; Sowell et al., 2004; Spear & Varlinskaya, 2005). While overall brain volume remains unchanged, the ongoing regressive and progressive processes of synaptic refinement and myelination during adolescence result in reduced gray matter and increased white matter volume by late adolescence (Giedd, 2004; Yakovlev & Lecours,

1967). Cortical gray matter loss during late childhood and adolescence is thought to be related to the pruning of excess neurons and begins primarily in dorsal parietal cortices, continuing anteriorly to the frontal cortex, then posteriorly to parietal, occipital, and finally temporal cortices (Gogtay et al., 2004), with decreases in dorsal prefrontal cortical volume by late adolescence (Sowell, Thompson, Tessner, & Toga, 2001). Gray matter loss during adolescence is also observed in subcortical structures such as the globus pallidus, caudate, putamen, thalamus, and nucleus accumbens (Giedd et al., 1996; Huttenlocher, 1990; Sowell, Thompson, Holmes, Jernigan, & Toga, 1999). Co-occurring increases in white matter during adolescence are associated with greater structural connectivity between brain regions (Barnea-Goraly et al., 2005; Giedd, Blumenthal, et al., 1999; Hüppi & Dubois, 2006; Jernigan & Gamst, 2005; Paus et al., 1999; Pfefferbaum et al., 1994; Sowell et al., 2004) and smoother, more efficient communication between frontal-subcortical brain regions (Luna & Sweeney, 2004). In particular, significant volume increases in the right internal capsule and left arcuate fasciculus suggest increased connectivity between regions associated with speech (Paus et al., 1999), and increased volume of the corpus callosum, the brain's largest white matter tract, suggest greater interhemispheric communication (Barnea-Goraly et al., 2005; Giedd, Castellanos, et al., 1999).

These extensive neural transformations during adolescence, in addition to neurochemical modifications in prefrontal regions and limbic systems, correspond to a range of cognitive, emotional, and behavioral changes that are hypothesized to contribute to adolescents' increased propensity for alcohol use (Casey, Jones, & Hare, 2008; Chambers & Potenza, 2003; Doremus-Fitzwater, Varlinskaya, & Spear, 2009; Spear &

Varlinskaya, 2005). The dopaminergic system is significantly reorganized in the adolescent brain, with dopamine activity substantially decreasing in the nucleus accumbens thus potentially increasing adolescents' propensity for actively seeking out risky and novel behaviors to compensate for this dopamine void (Spear, 2002). These increased motivational drives for novel experiences, coupled with less mature inhibitory capacity, could further influence an adolescent's susceptibility to engage in impulsive and risky behaviors like alcohol use (Casey, Duhoux, & Malter Cohen, 2010; Casey, Jones, et al., 2010; Casey, Jones, & Somerville, 2011; Chambers & Potenza, 2003).

Neural transformations during adolescence, including cortical thinning, increased fiber track efficiency, and neurochemical and hormonal changes, may also leave the brain more vulnerable to the neurotoxic effects of alcohol (Brown, Tapert, Granholm, & Delis, 2000; Clark & Tapert, 2008; Crews, Mdzinarishvili, Kim, He, & Nixon, 2006; Dahl, 2004; Monti et al., 2005; Spear, 2000; Spear & Varlinskaya, 2005; Squeglia, Jacobus, & Tapert, 2009; Tapert, Granholm, Leedy, & Brown, 2002). Given the considerable neurodevelopment that occurs during adolescence, understanding the effect of neural insults incurred during this period is of essential importance.

Heavy Drinking during Adolescence Is Linked to Neurocognitive Abnormalities

While the adult literature has consistently shown adverse effects of heavy substance use on physical and psychological well being (Cunha-Oliveira, Rego, & Oliveira, 2008; Oscar-Berman & Marinkovic, 2003, 2007; Vik, Cellucci, Jarchow, & Hedt, 2004), research has only begun to explore potentially negative neuropsychological sequelae associated with alcohol use during adolescence. Cross-sectional neuropsychological studies have shown that alcohol use during adolescence is associated

with decrements in visuospatial performance (Brown et al., 2000; Giancola, Mezzich, & Tarter, 1998; Sher, Martin, Wood, & Rutledge, 1997; Tapert & Brown, 1999), sustained attention and speeded information processing (Tapert & Brown, 2000; Tarter, Mezzich, Hsieh, & Parks, 1995; Thoma et al., 2011), verbal and non-verbal learning and retrieval (Brown et al., 2000), language competence and academic achievement (Tarter et al., 1995), and overall reduction in keeping up with age expectations (Tapert & Brown, 1999, 2000). Poorer performance on tests of executive functioning, particularly during tasks associated with planning, abstract reasoning, and problem solving, have also been found (Giancola, Shoal, & Mezzich, 2001; Moss, Kirisci, Gordon, & Tarter, 1994; Thoma et al., 2011).

To disentangle premorbid factors from true alcohol-related deficits, longitudinal studies examining adolescent heavy substance use have been conducted. Deficits on tasks of attention and visuospatial functioning have been found in adolescents treated for substance use disorders who reported continued heavy drinking and greater alcohol hangover or withdrawal symptoms than adolescents who remained abstinent over the follow-up period (Tapert & Brown, 1999; Tapert et al., 2002). Squeglia et al., (2009) replicated these findings in a prospective study characterizing adolescents (N=76, ages 12-14) neurocognition prior to initiating alcohol or drug use. Thirty-six adolescents (13 female) transitioned into moderate to heavy drinking, and were compared to demographically matched controls who remained non-users throughout the approximate 3-year follow-up period (n=40; 16 female). For girls, greater alcohol consumption in the months preceding the follow-up neuropsychological assessment was associated with significant relative worsening of visuospatial functioning, particularly on tests of

visuospatial memory. For boys, greater hangover symptoms in the year preceding follow-up testing were associated with a significant relative worsening of sustained attention. Results remained unchanged after controlling for recent substance use, suggesting decrements may persist over time. These preliminary longitudinal findings suggest that initiating moderately heavy alcohol use and incurring hangover during adolescence may adversely influence neurocognitive functioning, possibly due to ongoing neuromaturational processes that leave the brain more vulnerable to the deleterious effects of alcohol. The longitudinal nature of these studies lends support to the notion that negative neuropsychological sequelae may be attributed, at least in part, to substance use rather than predisposing factors. Future studies examining the extent of these functional deficits over time and their relationship to continued alcohol use and withdrawal symptoms is warranted. .

Heavy Drinking During Adolescence Is Associated with Brain Structure Abnormalities

Structural neuroimaging techniques have been utilized to elucidate the anatomical substrates underlying neuropsychological decrements associated with adolescent alcohol use. De Bellis and colleagues (2000) found significantly less left and right hippocampal volume in adolescents who met criteria for alcohol use and other co-occurring psychological disorders compared to controls, despite having no significant intracranial, cortical gray and white matter, amygdalae, and corpus callosum volume differences between groups. Smaller hippocampi volume was associated with earlier age of onset and longer duration of the alcohol use disorder (De Bellis et al., 2000). Given the high rate of co-occurring psychiatric disorders within this sample, Nagel and colleagues (Nagel, Schweinsburg, Phan, & Tapert, 2005) examined hippocampal volumes in a group of

adolescents without concomitant psychopathology to elucidate the effects of alcohol use only on hippocampi and found smaller left hippocampal volumes in alcohol-using adolescents compared to controls. Volume did not correlate with alcohol involvement in this sample, suggesting structural differences may have been premorbid. Another study using a similar sample found that increased alcohol use disorder symptoms were positively associated with greater right than left hippocampal asymmetry (Medina, Schweinsburg, Cohen-Zion, Nagel, & Tapert, 2007). Longitudinal studies are needed to disentangle the true effects of alcohol from predisposing factors on adolescent hippocampal volumes, as well as determine the neuropsychological correlates of hippocampal volume changes over time.

The frontal lobe is another area of particular interest in structural imaging studies of alcohol using youth, as this brain area is associated with higher-order, executive functioning, including problem solving, planning, impulse control, emotional regulation, integration of novel stimuli, motivation, and cognitive flexibility (Lezak, Howieson, Loring, Hannay, & Fischer, 2004). Lifetime heavy alcohol use has been associated with smaller prefrontal regions in adult alcoholics (Mechtcheriakov et al., 2007; Pfefferbaum, Sullivan, Mathalon, & Lim, 1997; Pfefferbaum, Sullivan, Rosenbloom, Mathalon, & Lim, 1998). Recent findings from adolescent populations suggest the prefrontal cortex may be more vulnerable to the effects of alcohol, as this area of the brain is continuing to develop into late adolescence (Sowell et al., 2001). In a sample of adolescents with co-occurring alcohol use and psychiatric disorders, De Bellis and colleagues (De Bellis et al., 2005) found that heavy drinking adolescents had significantly smaller prefrontal grey and white matter volumes than demographically matched controls. Medina and

colleagues (2008) found smaller anterior ventral prefrontal cortex volumes in a sample of adolescents who met criteria for an alcohol use disorder without co-occurring mood or attentional disorders, as compared to controls (Medina et al., 2008). A gender by alcohol interaction was present, with alcohol dependent females having smaller prefrontal cortex and white matter volumes than controls, and alcohol dependent males having larger prefrontal and white matter volumes than controls. These gender differences were also found in a study examining cortical thickness in non-clinical binge drinking adolescents (Squeglia, Sorg, Schweinsburg Dager, Wetherill, & Tapert, 2012), which found adolescent girls with a recent history of binge drinking tended to show thicker cortices in left frontal regions than demographically similar controls. Thicker frontal cortices were linked to worse visuospatial, inhibition, and attention performance. In contrast, adolescent boys with recent binge drinking showed thinner cortices in these same areas than light to non-drinking boys. These findings suggest that gender moderates the effect of adolescent alcohol use on prefrontal neuromaturation, even in adolescents who do not meet criteria for an alcohol use disorder, with females having more pronounced negative sequelae from continued drinking.

Heavy Drinking during Adolescence Is Associated with Poorer White Matter Integrity

White matter, comprised of fatty myelin-coated axons, is responsible for the communications between brain regions. Diffusion tensor imaging (DTI) can be used to examine the integrity of white matter tracts by measuring the coherence of white matter fibers. Greater white matter integrity has been associated with more efficient and speeded connectivity between brain regions and is related to better behavioral performance (Konrad, Vucurevic, Musso, Stoeter, & Winterer, 2009; Tuch et al., 2005).

In adult samples, chronic alcohol use has been associated with abnormal white matter volume and less organization of white matter tracts, particularly in frontal brain regions (Kril, Halliday, Svoboda, & Cartwright, 1997; Pfefferbaum, Adalsteinsson, & Sullivan, 2006; Pfefferbaum, Rosenbloom, Rohlfing, & Sullivan, 2009; Pfefferbaum & Sullivan, 2005; Pfefferbaum et al., 2000; Yeh, Simpson, Durazzo, Gazdzinski, & Meyerhoff, 2009). In adolescents with alcohol use disorders, preliminary studies have found that alcohol consumption during adolescence is associated with decrements in white matter volume (Medina et al., 2008) and integrity (McQueeny et al., 2009).

One DTI-derived measure of white matter integrity is fractional anisotropy, a measure of the directional coherence of white matter tracts (Basser & Jones, 2002; Lim & Helpern, 2002). Lower fractional anisotropy values (i.e., decreased white matter coherence) in the corpus callosum, an area of the brain responsible for interhemispheric communication, were found in a sample of adolescents with alcohol use disorders compared to non-using controls (Tapert, Theilmann, & Schweinsburg, 2003), which correlated with greater alcohol use, withdrawal, and recency of drinking. Widespread reductions in white matter integrity have also been found in adolescents who engaged in a binge drinking episode at least once in the three months prior to scanning. Binge drinkers showed decreased fractional anisotropy in 18 major fiber tract pathways, notably in the corpus callosum, superior longitudinal fasciculus, corona radiata, internal and external capsules, and commissural, limbic, brainstem, and cortical projection fibers. Directional coherence in 6 of the 18 regions was significantly related to greater lifetime hangover and/or higher estimated peak blood alcohol levels (McQueeny et al., 2009). Although cross-sectional, the substantial group differences were surprising given that the binge

drinkers had been drinking at subdiagnostic levels (i.e., did not meet diagnostic criteria for alcohol abuse or dependence).

Heavy Drinking during Adolescence Is Associated with Neural Functioning Abnormalities

Functional magnetic resonance imaging (fMRI) is used to investigate cognition by measuring subtle changes in blood oxygen level dependent (BOLD) signal during mental tasks or exposure to stimuli. BOLD signal is related to blood flow, and therefore, higher BOLD signals indirectly indicate regions of the brain with increased activation (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001; Ogawa, Lee, Kay, & Tank, 1990). More specifically, fMRI measures deoxygenated hemoglobin, which is paramagnetic and thus detectable by MRI without exogenous contrast agents. The more deoxygenated hemoglobin detected means less oxygenated hemoglobin which suggests, from single cell recording studies (Lee et al., 2010), less neural activity (Logothetis, 2002; Logothetis et al., 2001).

fMRI studies have been useful in exploring neural abnormalities in heavy drinking adolescents, as this technique is widely used, non-invasive and safe. Using a spatial working memory task, Tapert et al. (2004) found that adolescents with just one to two years of heavy drinking exhibited more activation in the parietal lobe and less activation in the occipital and cerebellar regions compared to light drinkers, despite equivalent performance on the task (Tapert, Schweinsburg, et al., 2004). This effect was particularly pronounced in adolescents reporting more withdrawal and hangover symptoms and greater lifetime alcohol use. These different activation patterns in the context of intact task performance raise the possibility of subtle neural reorganization

among heavy drinking adolescents early in their drinking trajectory. Moreover, in a study of young adult (ages 18-25) females who had engaged in four to five years of heavy drinking, Tapert and colleagues (2001) reported poorer performance on the same spatial working memory task during fMRI with subsequent decreased activation in the right superior and inferior parietal, right middle frontal, right postcentral, and left superior frontal cortices (Tapert et al., 2001). Unlike the previous study of adolescents, heavy drinking young adults performed worse on the spatial working memory task as well as other neuropsychological tests of working memory and executive functioning, which was in turn associated with greater alcohol-related withdrawal. Together, these findings may suggest that the brain may be able initially to compensate for subtle neural abnormalities associated with heavy alcohol exposure, while continued heavy alcohol use may interfere with the capacity for neural compensation.

More recent findings suggest that alcohol's effect on brain activation may have a gender-specific pattern (Squeglia, Dager Schweinsburg, Pulido, & Tapert, 2011). A sample of 40 binge drinkers (13 females, 27 males) and 55 controls (24 females, 31 males) ages 16 to 19 completed a spatial working memory task during fMRI. Significant binge drinking status x gender interactions were found ($p<.05$) in 8 brain regions spanning bilateral frontal, anterior cingulate, temporal, and cerebellar cortices. In all regions, female binge drinkers showed *less* spatial working memory activation than female controls, while male bingers exhibited *greater* spatial working memory response than male controls. For female binge drinkers, less activation was associated with poorer sustained attention and working memory performances. For male binge drinkers, greater activation was linked to better spatial performance. These findings coincide with studies

of adult alcoholics (Hommer, Momenan, Kaiser, & Rawlings, 2001; Hommer et al., 1996; Jacobson, 1986) suggesting that females may be more vulnerable to the neurotoxic effects of heavy alcohol use, here during adolescence, while males may be more resilient to the deleterious effects of binge drinking, particularly on tasks involving spatial functions (Caldwell et al., 2005; Squeglia et al., 2011).

Differential activation between drinkers and nondrinkers has also been found on tasks of verbal encoding, with drinkers showing less response in the right superior frontal, bilateral posterior parietal, and left hippocampal areas, but greater response in the occipital cortex, suggesting less utilization of working memory systems during verbal encoding for drinkers compared to nondrinkers (Schweinsburg, McQueeny, Nagel, Eyler, & Tapert, 2010). Youth may be able to compensate for alcohol-induced neuronal disturbances by recruiting additional brain regions through widespread neuronal activation, and therefore no behavioral disadvantages are evident during the early stages of alcohol use (Brown & Tapert, 2004). However, these reorganized activation patterns may suggest early disruption of neural functioning which could impair behavior over continued insults.

Understanding decreases in cerebral blood flow may help elucidate the metabolic changes underlying differences seen in functional brain activity in alcohol-using adolescents compared to nonusers. Reductions in blood flow to almost all brain regions have been found in adults with chronic alcoholism (Suzuki, Oishi, Mizutani, & Sato, 2002). In young alcohol dependent females, Clark and colleagues (Clark et al., 2007) found lower levels of blood flow in prefrontal and parietal regions (i.e., bilateral middle frontal gyri, left precuneus, right cingulate, and bilateral inferior parietal lobules)

compared to nondrinkers, with no regions of the brain showing greater blood flow. Thus, it is possible that some of the observed fMRI abnormalities could be attributable to brain blood perfusion deficits.

Supporting these fMRI data are event-related potential findings. Event-related potentials show underlying cognitive functioning using electrophysiological response to a stimulus. Frequent binge drinking during late adolescence has been associated with abnormal electrophysiological response during a visual working memory (VWM) task (Crego et al., 2009). Binge drinkers showed differential patterns of activation to task subcomponents, suggesting the need for greater attentional effort to perform the task. Furthermore, ability to determine relevant information appeared compromised, suggesting deficits in processes underlying working memory. Taken together, these fMRI, cerebral blood flow, and event-related potential studies suggest that adolescents who engage in heavy drinking may show abnormal brain activation compared to adolescents who abstain from alcohol use.

In a preliminary examination of the data proposed for this dissertation, cross-sectional fMRI BOLD response during a visual working memory task was examined (Squeglia, Pulido, Spadoni, Infante, & Tapert, June, 2009). Participants (ages 15-19; 55% female) were categorized as heavy drinkers (n=20; 39 drinks per month on average), and individually matched to non-users (n=20; 0 drinks per month) on age, gender, and family history of alcoholism. The visual working memory (VWM) task used in this study is the same task proposed for use for this dissertation and has 3 levels of working memory load. All data were processed and analyzed using Analysis of Functional NeuroImages (AFNI; afni.nimh.nih.gov/afni). *T*-tests compared heavy drinkers to non-users on BOLD response

to high-load relative to low-load trials of the VWM task. Heavy drinkers had less (corrected $p<.05$, clusters $\geq$ 908μL) BOLD response contrast to high relative to low working memory load trials than matched controls in left middle occipital and bilateral anterior cingulate cortex, but more response in right postcentral/inferior parietal, right middle frontal, right superior frontal, and bilateral medial frontal regions (see Figure 1). This is similar to previous findings in adolescents with alcohol use disorder seen on a spatial working memory task (Tapert, Schweinsburg, et al., 2004). Groups were statistically equivalent on accuracy and reaction time to each VWM condition, suggesting that heavy yet subdiagnostic drinking during adolescence may be associated with increased activation during high working memory loads in dorsal networks. Heavy drinkers showed less utilization of visual and attentional networks, yet greater reliance on the dorsal ("where") stream in comparison to non-drinkers (Pfefferbaum et al., 2001). These results may indicate early compensatory neural reorganization after just a few years of adolescent heavy drinking. However, because these results are cross-sectional, it is unclear if these abnormalities temporally followed the onset of alcohol intake. The proposed dissertation project will use longitudinal data to elucidate the chronicity of adolescent alcohol use and brain functioning over time.

Visual Working Memory Reliably Activates Brain Regions Linked to Heavy Drinking

The reasons for examining brain response to a VWM task are four-fold. First, working memory (i.e., the storage and manipulation of information) is an essential component of executive functioning and information processing (Lezak et al., 2004) and is critical to the development of logical thinking and reasoning (Mandler, 2007). Any deficits in working memory accrued from heavy drinking would have a substantial

negative effect on normal daily functioning of adolescents. Second, working memory continues to improve over the course of adolescence to early adulthood, with greater reliance on the right dorsolateral prefrontal cortex and bilateral parietal cortex during task performance (Crone, Wendelken, Donohue, van Leijenhorst, & Bunge, 2006). Therefore, adolescence is an ideal time to capture developmental differences between groups. Third, tasks of VWM evoke substantial cortical response, predominantly in prefrontal and parietal regions (Baker, Frith, Frackowiak, & Dolan, 1996; Courtney, Ungerleider, Keil, & Haxby, 1996; Friedman & Goldman-Rakic, 1994; Haxby, Petit, Ungerleider, & Courtney, 2000; Owen, Morris, Sahakian, Polkey, & Robbins, 1996; Petrides, Alivisatos, Evans, & Meyer, 1993), areas that appear particularly vulnerable to the effects of adolescent alcohol use (Schweinsburg, Schweinsburg, et al., 2005; Tapert, Schweinsburg, et al., 2004). Finally, the neural substrates of VWM have been comprehensively examined (Cabeza et al., 2004; Cohen et al., 1997; Courtney et al., 1996; Fougnie & Marois, 2006; Postle & D'Esposito, 1999; Smith & Jonides, 1999; Ungerleider, Courtney, & Haxby, 1998), and VWM can be easily probed by manipulating the number of items (i.e., load) presented to an individual (Luck & Vogel, 1997).

Proposed Model: Adolescent Heavy Drinking Impairs Neurocognitive Functioning over Time

Adolescence is a critical neurodevelopmental period associated with dramatic increases in rates of alcohol use and drinking to intoxication. Identifying the influence of adolescent alcohol drinking on brain functioning is important, as decrements incurred during ongoing neural maturation could have long-lasting effects on neural organization and cognitive functioning. Previous imaging studies have found subtle abnormalities in

brain response to working memory among adolescents with histories of heavy drinking (Schweinsburg et al., 2010; Schweinsburg, Schweinsburg, et al., 2005; Squeglia et al., 2011; Squeglia et al., June, 2009; Tapert, Pulido, Paulus, Schuckit, & Burke, 2004; Tapert, Schweinsburg, et al., 2004). As existing fMRI studies are cross-sectional, it is unclear if group differences predated the onset of drinking, or emerged as a result of drinking. The primary aim of this investigation is to prospectively examine BOLD response to a VWM task in adolescents who were first imaged prior to the onset of substance use, then transitioned into heavy drinking, versus youth who remained non-users. Follow-up analyses will examine if any neural abnormalities detected are associated with neuropsychological functioning. This research will help clarify the effects of alcohol use on brain functioning during adolescence, and aid in understanding whether deficits in VWM result from alcohol involvement, or are associated with premorbid factors (see Figure 2 for proposed model).

Hypotheses

Primary Aim: The primary aim of this study is to use longitudinal data on BOLD response to a VWM task to prospectively examine the influence of alcohol use on brain functioning, in adolescents first characterized prior to initiating any alcohol use.

Hypothesis 1: Initiators of moderate to heavy drinking during adolescence will show abnormalities in BOLD response to a VWM task, compared to adolescents who remained non-users, above and beyond baseline pre-substance use BOLD activation, despite similar performance on the VWM task. Specifically, moderate to heavy drinking during adolescence will be associated with increased BOLD response in right inferior parietal, right middle frontal, right superior frontal, and bilateral medial frontal regions,

and decreased BOLD response in the middle occipital gyrus, based on preliminary evidence (Squeglia et al., June, 2009).

Hypothesis 2: Abnormal BOLD response contrast in the hypothesized regions of interest (ROIs) will be linked to poorer performance on neuropsychological measures of visual memory, working memory, and attention, as attention underlies VWM processes (Cowan, 2001; Rensink, 2000a, 2000b, 2002). Specifically, increased BOLD response in right inferior parietal, right middle frontal, right superior frontal, and bilateral medial frontal regions, and decreased BOLD response in the middle occipital regions for heavy drinkers will be linked to poorer performance on Complex Figure 30-minute delay (Rey & Osterrieth, 1993a), WISC-III/WAIS-III Digit Span (Wechsler, 1991; Wechsler, 1997), and Digit Vigilance Test (Lewis, 1995). This hypothesis probes the neurobehavioral implications of activation differences proposed in Hypothesis 1, using more sensitive out-of-scanner tasks with wider ranges of task difficulty than the VWM task used during imaging.

METHODS

Participants

Participants (*N*=40; 20 heavy drinkers, 20 controls; baseline/pre-drinking and follow-up scan for each participant; 80 total scans) are part of a larger, ongoing neuroimaging study examining neurocognition in youths who are at-risk for developing substance use disorders (PI: Tapert, R01 AA13419; (Bava et al., 2010; McQueeny et al., 2009; Pulido, Anderson, Armstead, Brown, & Tapert, 2009; Spadoni, Norman, Schweinsburg, & Tapert, 2008; Squeglia, Spadoni, Infante, Myers, & Tapert, 2009; Tapert et al., 2007). See Tables 1-3 for demographic, substance use, and neuropsychological characteristics between groups at baseline and follow-up. Participants were recruited through flyers sent to all households of six middle schools in two San Diego area school districts and included a description of the study, major inclusion criteria, financial compensation ($170 for 5.5 hours), and contact information. Extensive screening and background information was obtained from the youth who responded to the ad, their biological parent, and one other parent or close relative. Advantages of recruiting through local schools included acquiring a representative sample that was motivated to participate and a lower chance of potentially confounding comorbid pathologies that are common in treatment-based recruitment sources. The study protocol was executed in accordance with the standards approved by the University of California, San Diego Human Research Protections Program (UCSD IRB approval #090269).

Baseline inclusion criteria. At baseline, participants were between ages 12 to 16 years, which corresponds to a time when many alcohol dependent young adults initially begin drinking. Further, synaptic refinement of frontal regions and gray matter pruning

are heightened between the ages of 12 and 16 years (Giedd, Blumenthal, et al., 1999; Sowell et al., 1999), making this an ideal time to capture developmental differences between groups. All participants had minimal experience with alcohol and drugs at their baseline assessment ($\leq$10 total days in their life in which drinking had occurred, with $\leq$2 drinks in a week; $\leq$1 lifetime experiences with marijuana and none in the past three months; $\leq$5 lifetime cigarette use; and no history of other intoxicant use). Youth with risk factors for drinking problems (i.e., family history of alcohol dependence, conduct disorder, and having tried alcohol before age 14) were over-represented in the sample.

Baseline exclusionary criteria. At baseline, adolescents were excluded for (1) prenatal alcohol (>2 drinks during a given week) or illicit drug exposure; (2) history of chronic medical illness; (3) any neurological (e.g., seizure disorder, migraines) or DSM-IV (APA, 1994) Axis I disorder other than oppositional defiant or conduct disorder; (4) head trauma or loss of consciousness (>2 minutes); (5) learning disabilities or mental retardation; (6) parental history of bipolar, psychotic, or antisocial personality disorder (ASPD); (7) colorblindness or non-correctable vision or hearing problems; (8) left handedness, as brain lateralization for these individuals differs from that of right-handed samples; (9) current use of medications potentially affecting the brain or cerebral blood flow (e.g. psychotropic medications); (10) premature birth (i.e., born prior to 35th gestational week); (11) pregnant on day of scanning; (12) arriving to the scan appointment intoxicated on alcohol or other drug (confirmed via breathalyzer and urinalysis); (13) inadequate comprehension of English, since this will limit ability to participate in the assessment process; (14) no resource person to verify use status; (15)

claustrophobia; (16) irremovable metal implements, and (17) adolescent and parent/guardian do not both provide informed assent and consent, respectively.

Measures

Screening. When youth or parents responded to the flyer, the Initial Youth and Parent Brief Screening was conducted, in which the study was briefly described, including the purpose, procedures, risks/benefits, and confidentiality. Parents and youth were verbally consented and then separately asked questions regarding exclusionary criteria, which were approved by UCSD IRB (e.g., child's age, handedness, irremovable metal implements, sensory problems, yes/no questions regarding child's substance use). Participants and parents were informed that all information provided is confidential within ethical and legal limits to facilitate disclosure. Informed consent and assent forms were mailed to potentially eligible families. When signed consents were received, a rigorous, detailed screening was conducted with the youth, either by phone or in person. Youth and parent were separately asked questions regarding demographic and background information, medication use, physical health, neurological history, previous head injuries, birth complications, family history of substance use, and personal substance use history. If exclusions were met, parents and youth were informed of their ineligibility and thanked for their time.

Eligible adolescents completed a more Detailed Youth Screening, using the Diagnostic Interview Schedule for Children 4.0 Predictive Scales (DPS; (Lucas et al., 2001; Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000) to facilitate excluding adolescents with probable psychiatric disorders. This measure ascertained information on past year and lifetime social phobia, separation anxiety, panic, generalized anxiety,

obsessive-compulsive disorder, major depression, mania, specific phobia, attention deficit hyperactivity disorder (ADHD), and alcohol and marijuana use disorder diagnoses. If parent and/or youth indicated probable diagnosis for any disorder other than a simple phobia, conduct disorder, or oppositional defiant disorder, the participant was excluded.

The brief lifetime version of the Customary Drinking and Drug Use Record (CDDR; (Brown et al., 1998) was administered to obtain self-report on quantity and frequency of lifetime and recent (past 3-month) alcohol, tobacco, and other drug use. Youth typically provide valid self-reports of alcohol, cigarette, and other drug use (Winters, Stinchfield, Henly, & Schwartz, 1990-91), but to maximize accuracy, a comfortable context for the interview was provided, teens and parents were assured confidentiality of substance use information, and corroborating information from parents and other biological sources was discussed and collected.

The Family History Assessment Module (FHAM; (Rice et al., 1995) gathered information on family history of substance use disorders, depression, anxiety, bipolar, psychotic episodes, and ASPD in first and second degree relatives. ASPD in parent is exclusionary due to its association with confounding factors such as disruptive family environment, child abuse, and abnormal brain activation (Hill, Shen, Lowers, & Locke, 2000; Ichiyama, Zucker, Fitzgerald, & Bingham, 1996; Tapert, Schweinsburg, et al., 2004).

The Structured Clinical Interview (SCI; (Brown, Myers, Mott, & Vik, 1994) ascertained psychosocial functioning (e.g., academic functioning, extracurricular activities, social functioning, family characteristics, living arrangements) and health history. Detailed screening typically took 60-90 minutes and all participants were paid

$20. If exclusion was met, youth were informed of their ineligibility and thanked for their time.

If the youth remained eligible, a biological parent was administered a Parent Detailed Screen, including the SCI (Brown et al., 1994) to assess fetal and infant development, childhood behavior, psychosocial functioning (i.e., academic, extracurricular, and social activities of youth), family characteristics, and parent education and occupation. To improve youth psychopathology reports, the parent version of the DPS administered questions regarding social phobia, separation anxiety, panic, generalized anxiety, obsessive-compulsive disorder, ADHD, depression, mania, alcohol, and marijuana use disorder diagnoses. The teen was excluded if either youth or parent report indicated probable psychiatric disorder other than conduct disorder or oppositional defiant disorder.

Family history information was collected from both the primary and the secondary parent (or, in <7% of cases, another close relative). The Computerized Diagnostic Interview Schedule for DSM-IV (CDIS; (Robins, Cotter, Bucholz, & Compton, 1996) modules of mania, schizophrenia, ASPD, alcohol dependence and abuse, and drug dependence and abuse, and the FHAM (Rice et al., 1995) were administered to both parents. The CDIS was used to assess family history of alcohol dependence and other substance use disorders, ASPD, bipolar disorder, and schizophrenia for all of the child's biological first and second-degree relatives (i.e., the child's other parent, siblings, maternal and paternal aunts, uncles, and grandparents). Socioeconomic status was determined with the Revised Socioeconomic Index of Occupational Status (Stevens & Featherman, 1981). The primary parent was paid $20 (approx. 1 - 1 ½ hours) and the

other parent $20 (approx. 1 hour) for completing the detailed parent screen.

Youth diagnoses were considered present if either the parent report or youth report indicated probable diagnosis. Parent ASPD, bipolar, or schizophrenia diagnoses were considered present if either parent's report meets criteria. If parent alcohol use disorder diagnoses were contradictory, permission was sought to collect FHAM data from an additional relative. Parent and adolescent reports of conduct disorder criteria tend to have good agreement (kappa = .79) (Cantwell, Lewinsohn, Rohde, & Seeley, 1997). These decisions were reviewed in weekly consensus meetings. All screening procedures were completed by phone or in-person according to the interviewee's preference. Based on the current study, 12% remain eligible after all screening interviews. The most common exclusionary criteria were youth psychiatric diagnoses and unavailability of both one biological parent and another close biological relative.

Neuropsychological testing. Measures of working memory, attention, visuospatial functioning, executive functioning, disinhibition, learning and memory, language, and academic achievement were administered at baseline and each follow-up. The neuropsychological battery lasted ~3 hours, and were conducted within a week of each scan session by a trained, reliable bachelors-, masters-, or Ph.D.-level psychometrician. See Table 3 for specific neuropsychological tests examined in this study.

Substance use. At each follow-up, the CDDR obtained quantity and frequency of past year and past 3-month alcohol, tobacco, and other drug use, withdrawal/hangover symptoms, and endorsement of abuse and dependence criteria. The Timeline Followback (TLFB; (Sobell & Sobell, 1992) assessed substance use quantity and frequency for the 30 days prior to the scan session. Temporal cues (e.g., holidays) were used to aid recall, and

a parent report of youth substance use was used as collateral evidence. The Hangover Symptoms Scale (HSS; (Slutske, Thomas M. Piasecki, & Hunt-Carter, 2003) was given at each follow-up to participants who endorsed any drinking in the past year. This measure provided severity ratings on 13 hangover symptoms experienced in the past 12 months (e.g., "Within the past 12 months when I drank alcohol, I experienced a headache the next morning"), with response options ranging from "never" (1) to "every time I drank alcohol" (5). Self-report of alcohol and other drug use was verified with Breathalyzer and urine toxicology screens.

Potential covariates. *Youth Self Report* (YSR; (Achenbach & Rescorla, 2001) provided a youth report and the *Child Behavior Checklist* (CBCL; (Achenbach & Rescorla, 2001) provided a parent report on level of adolescent psychopathological syndromes (e.g., internalizing and externalizing behaviors). The *Pubertal Development Scale* (Petersen, Crockett, Richards, & Boxer, 1988) provided a reliable and valid 5-item self-report measure of pubertal maturation, one item for females indicates the first day of the last menstrual cycle. The Pubertal Development Scale correlates significantly with physician ratings and Tanner Sexual Maturation Scale self-ratings (Miller, Tucker, Pasch, & Eccles, 1988, March). Between-group differences in pubertal development could account for developmental differences in brain activation; therefore, pubertal staging was important to measure as a covariate for subsequent data comparison.

State measures. The following measures were collected at the time of each scanning session. Current level of depression was assessed with the *Beck Depression Inventory-II* (BDI; (Beck, Steer, & Brown, 1996), which has been validated with 12 to 16 year-olds (Steer, Geetha, Ranieri, & Beck, 1998). The state portion of the *Spielberger*

State-Trait Anxiety Inventory (STAI; (Spielberger, Gorsuch, & Lushene, 1970) was administered to assess anxiety and ensure that youth were not experiencing any nervousness that could influence fMRI results, as anxiety has been associated with altered cerebral metabolism (Harris & Hoehn-Saric, 1995). The *Karolinska Sleepiness Scale* (KSS; (Åkerstedt & Gillberg, 1990) assessed alertness before and after scanning.

Procedures

Imaging. All imaging data used in this dissertation were collected from the 3T GE CXK4 short bore Excite-2 MR system with an 8-channel phase-array head coil at the UCSD Keck FMRI Center. Eight high bandwidth receivers for ultra-short TR times reduce signal distortions and signal dropout. Scan sessions involved the following:

1. Scout scans (10 seconds) assured good head placement and slice selection covering the whole brain.
2. MRI. The high-resolution *3d* T1-weighted sequence permitted volumetric analyses of white matter, gray matter, and CSF and tracing brain ROIs. A sagittally acquired spoiled gradient recalled sequence (FOV 24 cm, 256x256x192 matrix, .94x.94x1 mm voxels, 176 slices, TR=20 ms, TE=4.8 ms; flip angle 12˚, 7:26 minutes) was used.
3. Field map acquisitions employed 2 different echo times to assess field inhomogeneities and signal distortions under the same parameters as echo-planar images are acquired. This information was applied to the FMRI acquisitions to minimize warping and signal dropouts (~4 minutes total).
4. fMRI. BOLD signal was measured with T2*-weighted axially acquired echo-planar imaging sequences (FOV=24 cm, 64x64 matrix, 3.75x3.75x3.8 mm voxels,

32 slices, TE=30 ms, flip angle 90˚, ramped bandwidth 250 KHz). Task stimuli were back projected from a laptop to a screen at the foot of the scanner bed visible via an angled mirror attached to the head coil. Accuracy and reaction time data were logged with a fiber-optic response box (Current Designs, Pittsburgh, PA).

Task. Participants were administered the VWM task (Paulus, Tapert, Pulido, & Schuckit, 2006; Tapert, Pulido, et al., 2004) (see Figure 4) during fMRI acquisition. Each trial consisted of an array of 2, 4, or 6 colored dots briefly (100 ms) presented against a gray background. Because previous research has shown that 3 to 4 different items can be held simultaneously in VWM (Luck & Vogel, 1997), this task uses the 2-dot condition as the low capacity condition, 4-dot as mid-capacity condition, and 6-dot as high capacity condition. After a 1000 ms delay, the subsequent trial (2000 ms) included the same number of dots presented in the same location and were either the same color-array or one color different. For each trial, the subjects pressed button "1" if the color displays were the same and "2" if they differed. This was followed by a 500 ms timeout; 50% of the trials had identical color arrays, while 50% had a one color dot difference. Each subject completed 30 trials of each type (2, 4, or 6 dots) presented randomly, in addition to 69 null trials of 2000 ms each interspersed to provide an optimized fast-event related sequence. The task lasted 8 minutes and 32 seconds. The signal contrast during the 6-dot array relative to the 2-dot array (high capacity minus low capacity condition) was used as a measure of differential BOLD response. Participants who perform at or below chance level (50%) on the 2-dot (i.e., low-capacity/easy condition) were removed from analyses, as it is assumed that these individuals were not actively engaged in the task.

Follow-up procedures. Participants were followed annually, using rigorous

follow-up procedures (Kleschinsky, Bosworth, Nelson, Walsh, & Shaffer, 2009; Twitchell, Hertzog, Klein, & Schuckit, 1992). Each year after the baseline neuropsychological and imaging assessments were completed, participants were contacted by phone and administered a series of questionnaires assessing current substance use and psychiatric functioning. Those who met criteria for heavy or moderate substance use (see Figure 3) were invited to return and complete assessments (see Measures section).

Matching. Heavy drinkers and controls were matched on baseline and follow-up age and pubertal development, years since baseline, gender, family history of alcohol and substance dependence, socio-economic status, and internalizing/externalizing behavior (see Table 1).

Data Analysis

Image processing. Data were processed and analyzed using Analysis of Functional NeuroImages (AFNI) (Cox, 1996). Artifact and aberrant signal levels were examined in each repetition of each slice using an automated program developed by the UCSD Laboratory of Cognitive Neuroimaging. Motion in time series data were corrected by registering each acquisition to the maximally stable base volume with an iterated least squares algorithm (Cox & Jesmanowicz, 1999) to estimate 3 rotational and 3 displacement parameters for each participant. An output file specifying adjustments made controls for spin history effects (Friston, Williams, Howard, Frackowiak, & Turner, 1996) in analyses if no significant task-correlated motion was found. To evaluate task-related motion, the reference vector was correlated with the 6 motion parameters for each dataset. Datasets with significant task-correlated or bulk motion were excluded from

analyses. Two trained raters then scanned the time series *en cine* to omit any remaining repetitions with visually discernible motion. If more than 15% of repetitions in a task were discarded, the run was not used.

Deconvolution was conducted on time series data with a reference function that convolved the behavioral stimuli with a hemodynamic response model (Bandettini, Jesmanowicz, Wong, & Hyde, 1993), while covarying for linear trends and motion correction and ignoring the first 3 repetitions, resulting in a functional image in which every voxel contains a fit coefficient representing the change in signal across behavioral conditions, as well as % signal change and threshold statistics. Standardization transformations were made for each high-resolution anatomical image (Talairach & Tournoux, 1988), and functional datasets were warped in accordance to manage individual anatomical variability. Functional data were resampled into isotropic voxels (3 mm^3), and a spatial smoothing Gaussian filter (full-width half maximum 5 mm) was applied to minimize the influence of individual anatomic variability. Co-registration of structural images to functional images was performed with a mutual information registration program (Cox & Jesmanowicz, 1999) that robustly handles images with different signal characteristics and of different spatial resolutions. ROI masks were created for each hypothesized ROI using the brain atlases available in AFNI (Talairach & Tournoux). Masks were applied to the 6-dot (high working memory load) vs. 2-dot (low working memory load) contrast to extract a fit coefficient averaged across the ROIs, which was then imported to SPSS ("SPSS for Windows. Rel. 18.0.0. 2009. Chicago: SPSS Inc.,") for each ROI for each participant.

BOLD signal stability. While some studies have found high test-retest BOLD

signal reliability (Aron, Gluck, & Poldrack, 2006; Fernandez et al., 2003; Friedman et al., 2008; Specht, Willmes, Shah, & Jancke, 2003), others have reported considerable within-subject variation in BOLD signal change across scan sessions (Marshall et al., 2004; Tjandra et al., 2005; Zandbelt et al., 2008). Because questionable reliability threatens power and robustness of findings, several steps were taken to limit the influence of factors known to interfere with BOLD signal stability. Brain maturation is expected over the approximately three year follow-up (Bava et al., 2010; Klingberg, 2006; Schweinsburg, Nagel, et al., 2005), and could account for variance in BOLD signal change. Therefore, age and pubertal development were matched at baseline and follow-up for subjects to reduce the likelihood of maturation effects. Other less expected variation in BOLD signal stability could be accounted for by fluctuations in subject state factors like alertness, anxiety (Bishop, Duncan, & Lawrence, 2004; Harris & Hoehn-Saric, 1995), and effort (Specht et al., 2003). Anxiety and alertness were measured using the STAI and KSS, respectively, and effort was determined by each subject's performance on the 2 dot (i.e., low working memory load/easy) condition. It was assumed that individuals who performed at or below chance level were not actively engaged in the task and were removed from analyses.

Motion can interfere with BOLD stability, and therefore, extensive quality assurance was completed on all scan data, including visual inspection of every repetition in the task by two trained raters. If more than 15% of repetitions in a task were censored, the run was not used. Scanner drift could also interfere with reproducibility of the BOLD signal (Gunter et al., 2009), as well as changes in physiological factors, including respiration and cerebral blood flow (Menon, 2002; Petridou, Schäfer, Gowland, &

Bowtell, 2009; Tomasi & Caparelli, 2007). Physiological measures (e.g., respiration, pulse) were not acquired during scanning; therefore, future studies employing arterial spin labeling will be essential in disentangling physiological changes from true activation differences.

Because the stability of the BOLD signal has been brought into question, this study also examined fMRI BOLD response within each scan session on the 3T scanner where the data for this project was acquired. fMRI assessments were conducted with control subjects (i.e., individuals who meet all project eligibility criteria and have no significant substance use throughout the period of study) at baseline and follow-up (*n*=24; 48 scans) using the same VWM protocol at both time points. Intraclass correlation coefficients (ICC) were used to determine adequate within-session (i.e., 1st half vs. 2nd half scan session) BOLD signal reliability in all brain regions, including frontal, parietal, temporal, occipital, and subcortical areas. Analyses were completed in R statistics (R Development Core Team, 2009), using a locally created script (Brown et al., 2011) that calculates ICCs with restricted maximum likelihood for each voxel. For each voxel, the following were computed: (1) variability between subjects, (2) variability within the session (i.e., 1st vs. 2nd half of VWM task), and (3) unexplained variance (e.g., motion, artifact, random sources of "noise"). ICCs were calculated by dividing subject variance by the combined subject, run, and residual variance. Cichetti and Sparrow (1981) criteria were used for interpretation: <0.40 poor; 0.40–0.59 fair; 0.60–0.74 good; >0.74 excellent. These analyses were completed to assure the task was producing reliable BOLD response contrast values within sessions, and the results were used to interpret subsequent findings.

Hypothesis Testing

Hypothesis 1: *Initiators of moderate to heavy drinking during adolescence will show abnormalities in BOLD response to a VWM task, compared to adolescents who remained non-users, above and beyond baseline pre-substance use BOLD activation, despite similar performance on the VWM task. Specifically, moderate to heavy drinking during adolescence will be associated with increased BOLD response in right inferior parietal, right middle frontal, right superior frontal, and bilateral medial frontal regions, and decreased BOLD response in the middle occipital gyrus, based on preliminary evidence (Squeglia et al., June, 2009).*This hypothesis was tested in a repeated measures ANOVA with time (baseline and follow-up) as the within-subject factor, group (heavy drinker versus control) as the between-subjects factor, and subjects as a random factor to determine main effects of follow-up drinking status and time, as well as the interaction between drinking status and time on VWM high load relative to low load BOLD response contrast (6 vs. 2 dot), averaged across each ROI. Based on previous research (Squeglia et al., June, 2009; Tapert, Pulido, et al., 2004), it was hypothesized that adolescents who had transitioned into heavy drinkers would show greater BOLD response contrast in: (1) right inferior parietal, (2) right middle frontal, (3) right superior frontal, and (4) bilateral medial frontal regions, and decreased BOLD response in the (5) middle occipital gyrus, as compared to continuous non-drinkers, and in relation to their baseline activation (i.e., a group x time interaction). A *t*-test was used to determine differences on VWM task accuracy and reaction time between drinking groups. It was hypothesized that there would be no differences between groups on VWM task performance (accuracy and reaction time), suggesting compensatory neural reorganization rather than performance

deficit-driven activation differences. *Whole brain analyses.* An exploratory whole brain analysis determined if brain regions other than the ROIs might show significant BOLD response contrast change over time with drinking. AlphaSim (Ward, 2000) determined that activations comprised of at least 96 contiguous voxels (i.e., 2592 μL), each showing an effect at $p<.05$, would be considered a significant cluster size for these analyses to maintain family-wise (i.e., brain-wise) alpha of .05. A repeated measures group x time ANOVA compared heavy drinkers to non-users on BOLD response to high-load relative to low-load trials (i.e., 6 dot vs. 2 dot) of the VWM task.

Premorbid activation differences. An independent samples *t*-test was used to compare baseline data points between groups to determine differences in brain activation between adolescents who transition into heavy drinking compared to those who remain abstinent.

Covariates. Demographic and baseline characteristics that differed between groups were considered as a potential covariate when examining the association between the transition to heavy drinking and BOLD response. Baseline alcohol use (average lifetime uses: controls=0.5; heavy drinkers=1.5) and follow-up lifetime marijuana episodes (average lifetime uses: controls=0.2; heavy drinkers=84) significantly differed between groups and were treated as covariates in all analyses. Parental salary also significantly differed between groups; however this variable was not used as a covariate because the average salaries for both groups was well above average national income levels (average: controls=$109K; heavy drinkers=$171K) and represent similar economic classes (i.e., upper middle class). Furthermore, overall SES was similar between groups, as well as parental education.

Hypothesis 2: *Abnormal BOLD response contrast in the hypothesized regions of interest (ROIs) will be linked to poorer performance on neuropsychological measures of visual memory, working memory, and attention, as attention underlies VWM processes (Cowan, 2001; Rensink, 2000a, 2000b, 2002). Specifically, increased BOLD response in right inferior parietal, right middle frontal, right superior frontal, and bilateral medial frontal regions, and decreased BOLD response in the middle occipital regions for heavy drinkers will be linked to poorer performance on Complex Figure 30-minute delay (Rey & Osterrieth, 1993a), WISC-III/WAIS-III Digit Span (Wechsler, 1991; Wechsler, 1997), and Digit Vigilance Test (Lewis, 1995). This hypothesis probes the neurobehavioral implications of activation differences proposed in Hypothesis 1, using more sensitive out-of-scanner tasks with wider ranges of task difficulty than the VWM task used during imaging.*

This hypothesis probes the neurobehavioral implications of activation differences seen in Hypothesis 1, using more sensitive out-of-scanner tasks with wider ranges of task difficulty than the VWM task used during imaging. Hierarchical linear regressions examined if changes in BOLD response in regions that show a group x time interaction predicted follow-up neuropsychological performance on VWM and attention tasks for heavy drinkers (n=20). A composite score of neuropsychological tasks of visual memory, working memory, and sustained attention was created by averaging z-scores for Complex Figure 30-minute delay (Rey & Osterrieth, 1993b), WISC-III Digit Span (Wechsler, 1991), and the Digit Vigilance Test (Lewis, 1995). BOLD response signal change was created by subtracting each subject's baseline fit coefficient from his or her follow-up fit coefficient in each of the ROIs. Hierarchical regressions were used, with follow-up

neuropsychological performance composite score as the dependent variable, covariate (i.e., baseline alcohol use, follow-up lifetime marijuana use) on Block 1, and BOLD response signal change for each ROI on Block 2. The $R^2\Delta$ for the second step was interpreted to ascertain the degree to which alcohol-related changes in BOLD activation were associated with follow-up neuropsychological performance, above and beyond covariates. Bonferroni correction (p=.01) were used to maintain a family-wise alpha of p=.05, thereby protecting against Type I error rate. For significant ROIs, follow-up regression analyses were used to determine which neuropsychological measures in the composite score were significantly related to changes in BOLD response.

RESULTS

Task Performance

Task performance data were available for 19/20 controls and 18/20 heavy drinkers at baseline, and 18/20 controls and 20/20 heavy drinkers at follow-up. As expected, there was a significant time effect (*ps*<.05), for 2- and 6-dot accuracy and reaction time such that controls and heavy drinkers were more accurate and responded more quickly during the follow-up compared to their baseline accuracy and response times. At baseline, heavy drinking transitioners performed slightly faster (*p*<.05) on the 2-dot condition than controls. No other between group differences were observed at baseline or follow-up.

Continuous controls. At baseline, average accuracy for controls was 91% (range: 71-100%) on the 2-dot and 78% (range: 55-97%) on the 6-dot trial, and average reaction time was 2597 ms (range: 2050-2872 ms) for 2-dot and 2714 ms (range: 2110-2960 ms) for 6-dot. At follow-up, average accuracy for controls was 96% (range: 84-100%) on 2-dot and 84% (range: 67-97%) on 6-dot trials, and average reaction time was 2214 ms (range: 2035-2492 ms) for 2-dot and 2369ms (range: 2150-2686 ms) for 6-dot.

Heavy drinkers. At baseline, average accuracy for future heavy drinkers was 91% (range: 74-100%) on 2-dot and 79% (range: 65-94%) on 6-dot trials, and average reaction time was 2436ms (range: 2018-2874 ms) for 2-dot and 2611ms (range: 2184-2966 ms) for 6-dot. At follow-up, average accuracy for heavy drinkers was 95% (range: 84-100%) on 2-dot and 80% (range: 52-90%) on 6-dot trials, and average reaction time was 2214ms (range: 2081-2428 ms) for 2-dot and 2406 (range: 2170-2594 ms) for 6-dot (see Table 4).

Correlations between BOLD response and task performance. No correlations between task performance and 6- relative to 2-dot BOLD response contrast were observed at baseline or follow-up.

Activation to Task

BOLD response to the 6 vs. 2 dot condition was examined in all participants at baseline and follow-up (N=40; 80 total scans) to identify regions activated during the task (see Figure 5 for whole brain activation/deactivation patterns to 6 vs. 2 dot). As expected, occipital, parietal, and frontal regions were engaged to complete the task (see Figures 5 & 6 for whole brain and ROI activation).

Hypothesis 1

Region of interest findings. Significant group x time interactions were found in two of the five hypothesized ROIs, including right inferior parietal lobule (cluster size: 810 μL, p=.005; η^2=.23) and left medial frontal gyrus (cluster size: 1431 μL, p=.003; η^2=.19). These results held after controlling for baseline alcohol use and follow-up lifetime marijuana use, suggesting activation differences were robust to other substance use. Independent samples t-tests were used to probe significant group x time interactions. At baseline, heavy drinkers showed significantly less 6 vs. 2 dot activation than controls in both regions ($p\leq$.01). At follow-up however, heavy drinkers showed significantly increasing BOLD activation, while controls exhibited attenuated activation in both regions. At follow-up, heavy drinkers had greater activation than controls in the right inferior parietal lobule, which trended towards significant (p=.10). In the context of expected activation to the task over time (see Figure 6), adolescents who initiated heavy drinking over the follow-up period exhibited divergent BOLD response to the VWM task

compared to controls. Contrary to hypotheses, significant divergences in BOLD activation were not found in right middle frontal, right superior frontal, and middle occipital regions (see Figure 7 and Table 5).

Whole brain follow-up analyses. Whole-brain analyses examined if additional regions exhibited significant group x time interactions (>2538 μL, corrected $p<.05$). No additional significant group x time interactions were observed.

Hypothesis 2

Neuropsychological findings. Contrary to hypotheses, BOLD signal change was not related to performance on follow-up neuropsychological composite scores ($p<.01$). Exploratory analyses revealed no significant correlations between BOLD signal and individual neuropsychological test scores (both change scores over time and follow-up only scores; see Table 3 for specific neuropsychological tests examined).

Follow-up substance use and BOLD contrast correlates. For heavy drinkers (n=20), correlations between substance use variables and follow-up BOLD response contrast were examined for regions exhibiting significant group x time interactions. No significant correlations ($p<.05$) were found in the right inferior parietal lobule, but in the left medial frontal gyrus, lower BOLD response significantly correlated with greater past three month (r=-.50) and year (r=-.50) peak number of drinks, total amount drank in the past month (r=-.52), and total lifetime drinking days (r=-.43).

BOLD Signal Stability

In the regions where significant group x time interactions were observed, ICC values were examined to determine if these regions were reliably activating to the task. At both time points, there was high voxel-wise variability of ICC values in both regions

of interest. At baseline, ICC values within voxels ranged from .00 to .66 in the left medial frontal gyrus and .00 to .62 in the right inferior parietal lobule. At follow-up, values ranged from .00 to .78 in the left medial frontal gyrus and .00 to .45 in the right inferior parietal lobule. The left medial frontal gyrus had more reliable overall activation than the right inferior parietal lobule. Whole brain analyses show the task reliably activated several frontal, parietal, and occipital regions, as expected (see Figure 8).

DISCUSSION

This study prospectively examined the effects of alcohol use on brain activation in relatively healthy adolescent heavy drinkers and controls who were characterized (i.e., completed fMRI and neuropsychological testing) prior to initiating any alcohol use and were followed for approximately 3 years. As hypothesized, significant drinking status (i.e., group) x time interactions were observed in frontal and parietal regions. Specifically, pre-drinking differences in BOLD activation were found in adolescents who continued to abstain from alcohol compared to those who initiated heavy use at follow-up. At follow-up, adolescents who remained abstinent showed decreasing brain activation, consistent with expected neural development (Schweinsburg, Nagel, et al., 2005), while heavy drinkers showed increasing activation in the aforementioned areas.

Contrary to hypotheses, areas of increased activation did not relate to worsening neuropsychological test performance in the heavy drinkers, perhaps suggesting increased neural effort required to perform at the same level as non-using controls. The negative correlations between BOLD response and substance use variables in heavy drinkers at follow-up (i.e., greater substance use was related to lower BOLD response contrast) suggests the possibility that more intense drinking levels may be linked to failure to activate key regions to cognitive challenges, while more moderate heavy drinking is linked to over-activation that may compensate for subtle insults. Additional time points, larger sample size, and broader range of drinking patterns will be needed to verify this notion. These findings expound on previous studies showing parietal and frontal regions are most susceptible to adolescent heavy drinking (Schweinsburg, Schweinsburg, et al.,

2005; Tapert, Schweinsburg, et al., 2004), and help disentangle premorbid influences of adolescent substance abuse from post-drinking consequences.

The differential brain response between continuous controls and future heavy drinkers at baseline, before either group had significant substance exposure, may be a phenotypic marker for other risk factors related to the development of heavy drinking patterns during adolescence. At baseline, future heavy drinking adolescents exhibited greater 2-dot (less working memory load) than 6-dot condition (greater working memory) activation in the right inferior parietal lobule and left middle frontal gyrus. In contrast, controls exhibited greater 6- than 2-dot activation. Over time, controls had attenuated use of brain areas involved in visual working memory as expected (see Figure 6), while heavy drinkers had greater utilization of these regions.

The biological basis of differential neural activation at follow-up (i.e., increased activation in heavy drinkers) could be attributed to the disruption or slowing of synaptic pruning or myelination that typically occurs during adolescence. Synaptic pruning (i.e., the loss of unnecessary neural connections) creates more efficient and speeded information processing (Huttenlocher, 1990); therefore, alcohol-related disruption of synaptic pruning could contribute to deficient processing of information (Sullivan & Pfefferbaum, 2005), which would explain the region-specific over-activation of BOLD response (Karlsgodt et al., 2007; Kim et al., 2010; Manoach et al., 1999). Additionally, disruption of white matter myelination (i.e., axon ensheathment of fatty tissue that optimizes transmission of electrical signals) causes slowed neural propagation (Fields, 2008; Le Bihan et al., 2001; Schmithorst, Wilke, Dardzinski, & Holland, 2005), which could be apparent in the abnormal BOLD response patterns of the heavy drinkers.

Activation differences could also be a consequence of neural death or glial damage resulting from excessive glutamatergic hyperexcitability (Krystal et al., 2006). Preclinical studies have reported neuron and glia genesis during adolescence is inhibited during alcohol intoxication (Crews et al., 2006), primarily through the neurotoxic effects of increased oxidative stress and proinflammatory proteins (Crews & Nixon, 2009; Nixon, 2006). Glia may be even more sensitive to the effects of alcohol (Miguel-Hidalgo et al., 2002), with glial degeneration leading to deficient support and protection of neurons, further inhibiting neuronal development (Laming et al., 2000). Potentially, all abovementioned mechanisms (e.g., disrupted synaptic pruning and/or myelination, neuron and/or glial death) could interact to explain the aberrant brain activation patterns observed in alcohol-using adolescents versus controls.

Contrary to hypotheses, relationships between BOLD response contrast and the more sensitive, out-of-scanner neuropsychological test scores were not observed. This may suggest heavy drinkers are initially able to compensate for neural abnormalities by utilizing a greater number of brain regions (i.e., more diffuse activation) or increasing utilization (i.e., over-activation) of brain regions specific to the task (Brown & Tapert, 2004; Karlsgodt et al., 2007; Kim et al., 2010; Manoach et al., 1999). With continued drinking or other substance use, behavioral deficits may become more apparent over time (Squeglia, Spadoni, et al., 2009; Tapert et al., 2001; Tapert & Brown, 1999; Tapert et al., 2002).

The adolescents in this sample are relatively high functioning, from high socioeconomic status, have no current or past psychological or neurological disorders, have minimal, if any, current other substance use, and have limited alcohol-use histories

(average=93 lifetime alcohol use occasions for heavy drinkers). More prominent differences between controls and heavy drinkers might be found in adolescents with fewer resources, greater co-occurring pathology and other substance use, and longer, more pronounced histories of alcohol use.

Limitations

There are a few limitations to this study. Different follow-up durations were used across participants, ranging from 1.5 to five years; ideally, each individual would be examined after the same follow-up duration. Subjects were matched between groups on baseline and follow-up age and pubertal development, as well as follow-up duration to address this issue. While half of the heavy drinkers had used marijuana less than 10 times, some had engaged in mild to moderate amounts of marijuana use (average=84 lifetime uses for heavy drinkers). Adolescents who had used marijuana were included in the sample because of the lack of evidence showing negative effects of marijuana on neurocognitive functioning (Jacobus et al., 2009; Mahmood, Jacobus, Bava, Scarlett, & Tapert, 2010; Schweinsburg, Schweinsburg, Nagel, Eyler, & Tapert, 2011; Squeglia, Jacobus, et al., 2009) and to increase the generalizability of findings, as alcohol and marijuana use commonly co-occur in adolescents (Johnston et al., 2011).

fMRI BOLD response test-retest reliability has been questioned. High test-retest reliability on fMRI tasks has been found over short (e.g., one day) (Friedman et al., 2008) and long (e.g., one year) (Aron et al., 2006) intervals in adult populations. Pilot data show adequate test retest reliability (ICC >.70) for proposed ROIs in adolescents (Tapert et al., unpublished data). Additionally field mapped data were applied to the FMRI acquisitions to minimize warping and signal dropouts and reduce mislocalization errors, particularly

in frontal regions.

To further examine the reliability of the BOLD signal to the VWM task, within-session ICC analyses were conducted using control subjects (i.e., individuals who meet all project eligibility criteria and have no significant substance use throughout the period of study) at baseline and follow-up (n=24). The left medial frontal gyrus exhibited more reliable activation to the task than the right inferior parietal lobule. Therefore, replication of findings in right inferior parietal areas may be less likely than for left medial frontal areas. While regions showing significant group x time interactions were not found to be highly reliable in high relative to low working memory load BOLD response contrast values, there were several brain regions that showed good to excellent reliability (Cicchetti & Sparrow, 1981). Therefore, in regions where no significant group x time interactions were found, unreliability of the BOLD response is not likely a contributing factor.

No measures of cerebral blood flow were obtained during the scan sessions. Because resting perfusion can affect the magnitude of the BOLD response (Brown et al., 2003; Stefanovic, Warnking, Rylander, & Pike, 2006), it is possible that differences in brain blood flow could explain BOLD response abnormalities between heavy drinkers and controls (Clark et al., 2007). To minimize the likelihood of this, subjects were asked to refrain from caffeine, alcohol, and nicotine use the day of scan. Additionally, no scans were performed on subjects who use medications affecting the brain or cerebral blood flow (e.g., psychotropic medications) to reduce the likelihood of perfusion differences confounding results. The STAI was administered before each scan session to assess anxiety and ensure that youth were not experiencing any nervousness that could influence

fMRI results, as anxiety has been associated with altered cerebral metabolism (Harris & Hoehn-Saric, 1995). STAI scores were examined between groups, and anxiety level did not differ between controls or drinkers at baseline or follow-up, so it is unlikely that this factor played a role in results. fMRI BOLD response is just one of several indices used to measure brain-related changes during adolescence. Diffusion tensor imaging, volumetric analyses, and arterial spin labeling will be used in future studies to characterize the neural changes associated with heavy drinking during adolescence.

Females may be more vulnerable to the deleterious effects of heavy alcohol use during adolescence (Caldwell et al., 2005; Medina et al., 2008; Squeglia et al., 2011; Squeglia, Jacobus, et al., 2009; Squeglia et al., 2012). With the current sample size, we have limited power to examine gender-specific effects. Future studies with larger sample sizes are needed. Other factors (possibly genetic) need to be explored to elucidate the relationship between substance use and brain activation. Results from this study only generalize to adolescents who match the strict exclusion and inclusion criteria

Public Health Significance

Identifying the deleterious influence of alcohol use on the adolescent brain is important, as heavy drinking is common in youth and the brain is undergoing significant structural and functional changes, particularly in frontal regions. Any damage to these areas could have lasting negative social, academic, and occupational implications. Most studies examining the effect of alcohol use on the developing adolescent brain have been cross-sectional, undermining the ability to attribute deficits associated with heavy drinking to consequences of alcohol use or as preexisting risk factors that contributed to early initiation of use. This study was conducted to delineate if alcohol use during

adolescence influences the neural substrates underlying VWM, an essential component of information processing and executive functioning, and to examine if neural abnormalities were related to neuropsychological functioning. Differences in BOLD activation at baseline between continuous controls and future heavy drinker were found, suggesting pre-existing brain activation could increase adolescent's susceptibility to engage in heavy drinking.

The differential brain response between groups suggests potential utility of fMRI in predicting future substance use. Divergent activation over the follow-up (i.e., heavy drinkers increasing activation while controls show decreasing activation) suggests adolescents who initiate heavy drinking show less efficient and mature processing of information. While differences in brain activation did not correlate with neuropsychological functioning, continued heavy use over adolescence could result in both activation and behavioral differences. Negative consequences associated with drinking could affect large numbers of youth who engage in moderate to heavy levels of alcohol consumption. Less efficient processing of working memory and attention-related information could deter heavy drinking adolescents from academic and occupational success. By understanding the neurocognitive deficits that may arise from heavy alcohol use and identifying modifiable risk factors, the results from this study should contribute to the existing treatment and secondary prevention of alcohol use disorders. The long-term goals of this line of work are to (1) disseminate any findings through adolescent drinking prevention materials and public service campaigns, and (2) inform intervention and psychoeducational programs on how to optimally intervene with youth engaging in

heavy drinking, considering brain response and neurocognitive patterns linked to adolescent alcohol use.

REFERENCES

Achenbach, T. M., & Rescorla, L. A. (2001). *Manual for the ASEBA School-Age Forms & Profiles.* . Burlington, VT: University of Vermont , Research Center for Children, Youth, & Families.

Åkerstedt , T., & Gillberg, M. (1990). Subjective and objective sleepiness in the active individual. *International Journal of Neuroscience, 52*, 29-37.

APA. (1994). *Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV)*. Washington, DC: American Psychiatric Association.

Aron, A. R., Gluck, M. A., & Poldrack, R. A. (2006). Long-term test-retest reliability of functional MRI in a classification learning task. *NeuroImage, 29*, 1000–1006.

Baker, S. C., Frith, C. D., Frackowiak, R. S., & Dolan, R. J. (1996). Active representation of shape and spatial location in man. *Cerebral Cortex, 6*, 612-619.

Bandettini, P. A., Jesmanowicz, A., Wong, E. C., & Hyde, J. S. (1993). Processing strategies for time-course data sets in functional MRI of the human brain. *Magnetic Resonance in Medicine, 30*, 161-173.

Barnea-Goraly, N., Menon, V., Eckert, M., Tamm, L., Bammer, R., Karchemskiy, A., et al. (2005). White matter development during childhood and adolescence: a cross-sectional diffusion tensor imaging study. *Cerebral Cortex, 15*(12), 1848-1854.

Basser, P. J., & Jones, D. K. (2002). Diffusion-tensor MRI: theory, experimental design, and data analysis. *NMR in Biomedicine, 15*, 456–457.

Bava, S., Thayer, R., Jacobus, J., Ward, M., Jernigan, T. L., & Tapert, S. F. (2010). Longitudinal characterization of white matter maturation during adolescence. *Brain Research, 1327*, 38-46.

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). *Manual for the Beck Depression Inventory-2*. San Antonio, TX.: Psychological Corporation.

Bishop, S. J., Duncan, J., & Lawrence, A. D. (2004). State anxiety modulation of the amygdala response to unattended threat related stimuli. *Journal of Neuroscience, 24*, 10364-10368.

Brown, G. G., Eyler Zorrilla, L. T., Georgy, B., Kindermann, S. S., Wong, E. C., & Buxton, R. B. (2003). BOLD and perfusion response to finger-thumb apposition after acetazolamide administration: differential relationship to global perfusion. *Journal of Cerebral Blow Flow & Metabolism, 23*(7), 829-837.

Brown, G. G., Mathalon, D. H., Stern, H., Ford, J., Mueller, B., Greve, D. N., et al. (2011). Multisite reliability of cognitive BOLD data. *NeuroImage, 54*(3), 2163-2175.

Brown, S. A., Myers, M. G., Lippke, L., Tapert, S. F., Stewart, D. G., & Vik, P. W. (1998). Psychometric evaluation of the Customary Drinking and Drug Use Record (CDDR): A measure of adolescent alcohol and drug involvement. *Journal of Studies on Alcohol, 59*(4), 427-438.

Brown, S. A., Myers, M. G., Mott, M. A., & Vik, P. W. (1994). Correlates of success following treatment for adolescent substance abuse. *Applied & Preventive Psychology, 3*, 61-73.

Brown, S. A., & Tapert, S. F. (2004). Adolescence and the trajectory of alcohol use: basic to clinical studies. *Annals of the New York Academy of Sciences, 1021*, 234-244.

Brown, S. A., Tapert, S. F., Granholm, E., & Delis, D. C. (2000). Neurocognitive functioning of adolescents: Effects of protracted alcohol use. *Alcoholism: Clinical and Experimental Research, 24*(2), 164-171.

Cabeza, R., Daselaar, S. M., Dolcos, F., Prince, S. E., Budde, M., & Nyberg, L. (2004). Task-independent and task-specific age effects on brain activity during working memory, visual attention and episodic retrieval. *Cerebral Cortex, 14*(4), 364-375.

Cahalan, D., Cisin, I., & Crossley, H. (1969). *American drinking practices*. New Brunswick, NJ: Rutgers Center of Alcohol Studies.

Caldwell, L. C., Schweinsburg, A. D., Nagel, B. J., Barlett, V. C., Brown, S. A., & Tapert, S. F. (2005). Gender and adolescent alcohol use disorders on BOLD (blood oxygen level dependent) response to spatial working memory. *Alcohol & Alcoholism, 40*(3), 194-200.

Cantwell, D. P., Lewinsohn, P. M., Rohde, P., & Seeley, J. R. (1997). Correspondence between adolescent report and parent report of psychiatric diagnostic data. *Journal of the American Academy of Child and Adolescent Psychiatry, 36*(5), 610-619.

Casey, B. J., Duhoux, S., & Malter Cohen, M. (2010). Adolescence: what do transmission, transition, and translation have to do with it? *Neuron, 67*(5), 749-760.

Casey, B. J., Jones, R. M., & Hare, T. A. (2008). The adolescent brain. *Annals of the New York Academy of Sciences, 1124*(111-126).

Casey, B. J., Jones, R. M., Levita, L., Libby, V., Pattwell, S. S., Ruberry, E. J., et al. (2010). The storm and stress of adolescence: insights from human imaging and mouse genetics. *Developmental Psychobiology, 52*(3), 225-235.

Casey, B. J., Jones, R. M., & Somerville, L. H. (2011). Braking and accelerating of the

adolescent brain. *Journal of Research on Adolescence, 21*(1), 21-33.

Chambers, R. A., & Potenza, M. N. (2003). Neurodevelopment, impulsivity, and adolescent gambling. *Journal of Gambling Studies, 19*(1), 53-84.

Cicchetti, D. V., & Sparrow, S. A. (1981). Developing criteria for establishing interrater reliability of specific items: applications to assessment of adaptive behavior. *American Journal of Mental Deficiency, 86*(2), 127–137.

Clark, C. P., Brown, G. G., Eyler, L. T., Drummond, S. P., Braun, D. R., & Tapert, S. F. (2007). Decreased perfusion in young alcohol-dependent women as compared with age-matched controls. *American Journal of Drug and Alcohol Abuse, 33*(1), 13-19.

Clark, D. B., & Tapert, S. F. (2008). Introduction to alcohol and adolescent brain development. *Alcoholism: Clinical and Experimental Research, 32*(3), 373-374.

Cohen, J. D., Perlstein, W. M., Braver, T. S., Nystrom, L. E., Noll, D. C., Jonides, J., et al. (1997). Temporal dynamics of brain activation during a working memory task. *Nature, 386*(6625), 604-608.

Courtney, S. M., Ungerleider, L. G., Keil, K., & Haxby, J. V. (1996). Object and spatial visual working memory activate separate neural systems in human cortex. *Cerebral Cortex, 6*, 39-49.

Cowan, N. (2001). The magical number 4 in short-term memory: A reconsideration of mental storage capacity. *Behavioral and Brain Sciences, 24*, 87-185.

Cox, R. W. (1996). AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research, 29*, 162-173.

Cox, R. W., & Jesmanowicz, A. (1999). Real-time 3D image registration for functional MRI. *Magnetic Resonance in Medicine, 42*(6), 1014-1018.

Crego, A., Holguín, S. R., Parada, M., Mota, N., Corral, M., & Cadaveira, F. (2009). Binge drinking affects attentional and visual working memory processing in young university students. *Alcoholism: Clinical & Experimental Research, 33*(11).

Crews, F. T., He, J., & Hodge, C. (2007). Adolescent cortical development: A critical period of vulnerability for addiction. *Pharmacology Biochemistry and Behavior, 86*(2), 189-199.

Crews, F. T., Mdzinarishvili, A., Kim, D., He, J., & Nixon, K. (2006). Neurogenesis in adolescent brain is potently inhibited by ethanol. *Neuroscience, 137*(2), 437-445.

Crews, F. T., & Nixon, K. (2009). Mechanisms of neurodegeneration and regeneration in alcoholism. *Alcohol and Alcoholism, 44*(2), 115-127.

Crone, E. A., Wendelken, C., Donohue, S., van Leijenhorst, L., & Bunge, S. A. (2006). Neurocognitive development of the ability to manipulate information in working memory. *Proceedings of the National Academy of Sciences, 103*(24), 9315–9320.

Cunha-Oliveira, T., Rego, A. C., & Oliveira, C. R. (2008). Cellular and molecular mechanisms involved in the neurotoxicity of opioid and psychostimulant drugs. *Brain Research Reviews, 58*, 192-208.

Dahl, R. E. (2004). Adolescent brain development: a period of vulnerabilities and opportunities. *Annals of the New York Academy of Sciences, 1021*, 1-22.

De Bellis, M. D., Clark, D. B., Beers, S. R., Soloff, P. H., Boring, A. M., Hall, J., et al. (2000). Hippocampal volume in adolescent-onset alcohol use disorders. *American Journal of Psychiatry, 157*(5), 737-744.

De Bellis, M. D., Narasimhan, A., Thatcher, D. L., Keshavan, M. S., Soloff, P., & Clark, D. B. (2005). Prefrontal cortex, thalamus, and cerebellar volumes in adolescents and young adults with adolescent-onset alcohol use disorders and comorbid mental disorders. *Alcoholism: Clinical & Experimental Research, 29*(9), 1590-1600.

Doremus-Fitzwater, T. L., Varlinskaya, E. I., & Spear, L. P. (2009). Motivational systems in adolescence: Possible implications for age differences in substance abuse and other risk-taking behaviors. *Brain and Cognition*.

Durston, S., Hulshoff Pol, H. E., Casey, B. J., Giedd, J. N., Buitelaar, J. K., & van Engeland, H. (2001). Anatomical MRI of the developing human brain: What have we learned? *Journal of the American Academy of Child and Adolescent Psychiatry*(40), 1012–1020.

Fernandez, G., Specht, K., Weis, S., Tendolkar, I., Reuber, M., Fell, J., et al. (2003). Intrasubject reproducibility of presurgical language lateralization and mapping using fMRI. *Neurology, 60*, 969-975.

Fields, R. D. (2008). White matter in learning, cognition and psychiatric disorders. *Trends in Neuroscience, 31*(7), 361-370.

Fougnie, D., & Marois, R. (2006). Distinct capacity limits for attention and working memory: Evidence from attentive tracking and visual working memory paradigms. *Psychological Science, 17*(6), 526-534.

Friedman, H. R., & Goldman-Rakic, P. S. (1994). Coactivation of prefrontal cortex and inferior parietal cortex in working memory tasks revealed by 2DG functional mapping in the rhesus monkey. *Neuroscience, 14*, 2775-2788.

Friedman, L., Stern, H., Brown, G. G., Mathalon, D. H., Turner, J., Glover, G. H., et al.

(2008). Test-Retest and Between-Site Reliability in a Multicenter fMRI Study. *Human Brain Mapping, 29*(8), 958-972.

Friston, K. J., Williams, S., Howard, R., Frackowiak, R. S. J., & Turner, R. (1996). Movement-related effects in fMRI time-series. *Magnetic Resonance in Medicine, 35*(3), 346-355.

Giancola, P. R., Mezzich, A. C., & Tarter, R. E. (1998). Executive cognitive functioning, temperament, and antisocial behavior in conduct disordered adolescent females. *Journal of Abnormal Psychology, 107*, 629-641.

Giancola, P. R., Shoal, G. D., & Mezzich, A. C. (2001). Constructive thinking, executive functioning, antisocial behavior, and drug use involvement in adolescent females with a substance use disorder. *Experimental and Clinical Psychopharmacology, 9*(2), 215-227.

Giedd, J. N. (2004). Structural magnetic resonance imaging of the adolescent brain. *Annals of the New York Academy of Sciences, 1021*(77-85).

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., et al. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nature Neuroscience, 2*(861-863).

Giedd, J. N., Castellanos, F. X., Jeffries, N. O., Vaituzis, A. C., Liu, H., Blumenthal, J., et al. (1999). Development of the human corpus callosum: A longitudinal MRI study. *Progress in Neuropsychopharmacology and Biological Psychiatry, 23*, 571-588.

Giedd, J. N., Snell, J. W., Lange, N., Rajapakse, J. C., Casey, B. J., Kozuch, P. L., et al. (1996). Quantitative magnetic resonance imaging of human brain development: Ages 4–18. *Cerebral Cortex, 6*(551-560).

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., et al. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences, 101*, 8174-8179.

Gunter, J. L., Bernstein, M. A., Borowski, B. J., Ward, C. P., Britson, P. J., Felmlee, J. P., et al. (2009). Measurement of MRI scanner performance with the ADNI phantom. *Medical Physics, 36*(6), 2193-2205.

Harris, G. J., & Hoehn-Saric, R. (1995). Functional neuroimaging in biological psychiatry. In J. Panksepp (Ed.), *Advances in biological psychiatry* (pp. 113-160). Greenwich, CT: JAI Press.

Haxby, J. V., Petit, L., Ungerleider, L. G., & Courtney, S. M. (2000). Distinguishing the functional roles of multiple regions in distributed neural systems for visual working memory. *NeuroImage, 11*, 380-391.

Hill, S. Y., Shen, S., Lowers, L., & Locke, J. (2000). Factors predicting the onset of adolescent drinking in families at high risk for developing alcoholism. *Biological Psychiatry, 48*(4), 265-275.

Hommer, D. W., Momenan, R., Kaiser, E., & Rawlings, R. R. (2001). Evidence for a gender–related effect of alcoholism on brain volumes. *American Journal of Psychiatry, 158*, 198–204.

Hommer, D. W., Momenan, R., Rawlings, R., Ragan, P., Williams, W., Rio, D., et al. (1996). Decreased corpus callosum size among alcoholic women. *Archives of Neurology, 53*, 359–363.

Hüppi, P. S., & Dubois, J. (2006). Diffusion tensor imaging of brain development. *Seminars in Fetal and Neonatal Medicine, 11*, 489-497.

Huttenlocher, P. R. (1990). Morphometric study of human cerebral cortex development. *Neuropsychologia, 28*, 517-527.

Ichiyama, M. A., Zucker, R. A., Fitzgerald, H. E., & Bingham, C. R. (1996). Articulating subtype differences in self and relational experience among alcoholic men using structural analysis of social behavior. *Journal of Consulting and Clinical Psychology, 64*(6), 1245-1254.

Jacobson, R. (1986). The contributions of sex and drinking history to the CT brain scan changes in alcoholics. *Psychological Medicine, 16*, 547-549.

Jacobus, J., McQueeny, T., Bava, S., Schweinsburg, B. C., Frank, L. R., Yang, T. T., et al. (2009). White matter integrity in adolescents with histories of marijuana use and binge drinking. *Neurotoxicology & Teratology, 31*(6), 349-355.

Jernigan, T. L., & Gamst, A. C. (2005). Changes in volume with age–consistency and interpretation of observed effects. *Neurobiology of Aging, 26*(9), 1271-1274 (discussion 1275-1278).

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2011). *Monitoring the Future national results on adolescent drug use: Overview of key findings, 2010*. Bethesda, MD: National Institute on Drug Abuse.

Karlsgodt, K. H., Glahn, D. C., van Erp, T. G., Therman, S., Huttunen, M., Manninen, M., et al. (2007). The relationship between performance and fMRI signal during working memory in patients with schizophrenia, unaffected co-twins, and control subjects. *Schizophrenia Research, 89*(1-3), 191-197.

Kim, M. A., Tura, E., Potkin, S. G., Fallon, J. H., Manoach, D. S., Calhoun, V. D., et al.

(2010). Working memory circuitry in schizophrenia shows widespread cortical inefficiency and compensation. *Schizophrenia Research, 117*(1), 42-51.

Kleschinsky, J. H., Bosworth, L. B., Nelson, S. E., Walsh, E. K., & Shaffer, H. J. (2009). Persistence pays off: follow-up methods for difficult-to-track longitudinal samples. *Journal of Studies on Alcohol & Drugs, 70*(5), 751-761.

Klingberg, T. (2006). Development of a superior frontal-intraparietal network for visuo-spatial working memory. *Neuropsychologia, 44*(11), 2171-2177.

Konrad, A., Vucurevic, G., Musso, F., Stoeter, P., & Winterer, G. (2009). Correlation of brain white matter diffusion anisotropy and mean diffusivity with reaction time in an oddball task. *Neuropsychobiology, 60*(2), 55-66.

Kril, J. J., Halliday, G. M., Svoboda, M. D., & Cartwright, H. (1997). The cerebral cortex is damaged in chronic alcoholics. *Neuroscience, 79*(4), 983-998.

Krystal, J. H., Staley, J., Mason, G., Petrakis, I. L., Kaufman, J., Harris, R. A., et al. (2006). Gamma-aminobutyric acid type A receptors and alcoholism: intoxication, dependence, vulnerability, and treatment. *Archives of General Psychiatry, 63*(9), 957-968.

Laming, P. R., Kimelberg, H., Robinson, S., Salm, A., Hawrylak, N., Müller, C., et al. (2000). Neuronal-glial interactions and behaviour. *Neuroscience and Biobehavioral Reviews, 24*(3), 295-340.

Le Bihan, D., Mangin, J. F., Poupon, C., Clark, C. A., Pappata, S., Molko, N., et al. (2001). Diffusion tensor imaging: concepts and applications. *Journal of Magnetic Resonance Imaging, 13*(4), 534-546.

Lee, J. H., Durand, R., Gradinaru, V., Zhang, F., Goshen, I., Kim, D. S., et al. (2010). Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. *Nature, 465*(7299), 788-792.

Lewis, R. F. (1995). *Digit Vigilance Test*. Odessa, FL: Psychological Assessment Resources.

Lezak, M. D., Howieson, D. B., Loring, D. W., Hannay, H. J., & Fischer, J. S. (2004). *Neuropsychological Assessment* (4th ed.). New York: Oxford University Press.

Lim, K. O., & Helpern, J. A. (2002). Neuropsychiatric applications of DTI—a review. *NMR in Biomedicine, 15*, 587-593.

Logothetis, N. K. (2002). The neural basis of the blood-oxygen-level-dependent functional magnetic resonance imaging signal. *Philosophical Transactions of the Royal Society B: Biological Sciences, 357*(1424), 1003-1037.

Logothetis, N. K., Pauls, J., Augath, M., Trinath, T., & Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature, 412*(6843), 150-157.

Lucas, C. P., Zhang, H., Fisher, P. W., Shaffer, D., Regier, D. A., Narrow, W. E., et al. (2001). The DISC Predictive Scales (DPS): efficiently screening for diagnoses. *Journal of American Academy of Child & Adolescent Psychiatry, 40*(4), 443-449.

Luck, S. J., & Vogel, E. K. (1997). The capacity of visual working memory for features and conjunctions. *Nature, 390*, 279-281.

Luna, B., & Sweeney, J. A. (2004). The emergence of collaborative brain function: fMRI studies of the development of response inhibition. *Annals of the New York Academy of Sciences, 1021*, 296-309.

Mahmood, O. M., Jacobus, J., Bava, S., Scarlett, A., & Tapert, S. F. (2010). Learning and memory performances in adolescent users of alcohol and marijuana: interactive effects. *Journal of Studies on Alcohol and Drugs, 71*(6), 885-894.

Mandler, J. M. (2007). On the origins of the conceptual system. *The American Psychologist, 62*(8), 738-751.

Manoach, D. S., Press, D. Z., Thangaraj, V., Searl, M. M., Goff, D. C., Halpern, E., et al. (1999). Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. *Biological Psychiatry, 45*(9), 1128–1137.

Marshall, I., Simonotto, E., Deary, I. J., Maclullich, A., Ebmeier, K. P., Rose, E. J., et al. (2004). Repeatability of motor and working-memory tasks in healthy older volunteers: Assessment at functional MR imaging. *Radiology, 233*(3), 868-877.

McQueeny, T., Schweinsburg, B. C., Schweinsburg, A. D., Jacobus, J., Bava, S., Frank, L. R., et al. (2009). Altered white matter integrity in adolescent binge drinkers. *Alcoholism: Clinical and Experimental Research., 33*(7), 1278-1285.

Mechtcheriakov, S., Brenneis, C., Egger, K., Koppelstaetter, F., Schocke, M., & Marksteiner, J. (2007). A widespread distinct pattern of cerebral atrophy in patients with alcohol addiction revealed by voxel-based morphometry. *Journal of Neurology, Neurosurgery, and Psychiatry, 78*(6), 610-614.

Medina, K. L., McQueeny, T., Nagel, B. J., Hanson, K. L., Schweinsburg, A. D., & Tapert, S. F. (2008). Prefrontal cortex volumes in adolescents with alcohol use disorders: unique gender effects. *Alcoholism: Clinical & Experimental Research, 32*(3), 386-394.

Medina, K. L., Schweinsburg, A. D., Cohen-Zion, M., Nagel, B. J., & Tapert, S. F. (2007). Effects of alcohol and combined marijuana and alcohol use during adolescence on

hippocampal volume and asymmetry. *Neurotoxicology & Teratology, 29*, 141-152.

Menon, R. (2002). Postacquisition suppression of large-vessel BOLD signals in high-resolution fMRI. *Magnetic Resonance Imaging, 47*, 1-9.

Miguel-Hidalgo, J. J., Wei, J., Andrew, M., Overholser, J. C., Jurjus, G., Stockmeier, C. A., et al. (2002). Biological Psychiatry. *Glia pathology in the prefrontal cortex in alcohol dependence with and without depressive symptoms, 52*(12), 1121-1133.

Miller, C. L., Tucker, M. L., Pasch, L., & Eccles, J. S. (1988, March). Measuring pubertal development: A comparison of different scales and different sources. *Paper presented at the biennial meeting of the Society for Research in Child Development, Alexandria, VA.*

Monti, P. M., Miranda, R., Jr., Nixon, K., Sher, K. J., Swartzwelder, H. S., Tapert, S. F., et al. (2005). Adolescence: booze, brains, and behavior. *Alcoholism: Clinical & Experimental Research, 29*(2), 207-220.

Moss, H. B., Kirisci, L., Gordon, H. W., & Tarter, R. E. (1994). A neuropsychologic profile of adolescent alcoholics. *Alcoholism: Clinical & Experimental Research, 18*(159-163).

Nagel, B. J., Schweinsburg, A. D., Phan, V., & Tapert, S. F. (2005). Reduced hippocampal volume among adolescents with alcohol use disorders without psychiatric comorbidity. *Psychiatry Research: Neuroimaging, 139*(181-190).

Nixon, K. (2006). Alcohol and adult neurogenesis: roles in neurodegeneration and recovery in chronic alcoholism. *Hippocampus, 16*(3), 287-295.

Ogawa, S., Lee, T. M., Kay, A. R., & Tank, D. W. (1990). Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proceedings of the National Academy of Sciences of the United States of America, 87*(24), 9868-9872.

Oscar-Berman, M., & Marinkovic, K. (2003). Alcoholism and the brain: an overview, *Alcohol Research and Health* (Vol. 27, pp. 125-133).

Oscar-Berman, M., & Marinkovic, K. (2007). Alcohol: Effects on neurobehavioral functions and the brain. *Neuropsychology Review, 17*(3), 239-257.

Owen, A. M., Morris, R. G., Sahakian, B. J., Polkey, C. E., & Robbins, T. W. (1996). Double dissociations of memory and executive functions in working memory tasks following frontal lobe excisions, temporal lobe excisions or amygdalo-hippocampectomy in man. *Brain, 119*, 1597-1615.

Paulus, M. P., Tapert, S. F., Pulido, C., & Schuckit, M. A. (2006). Alcohol attenuates load-related activation during a working memory task: Relation to level of response to alcohol. *Alcoholism: Clinical and Experimental Research, 30*, 1363-1371.

Paus, T., Zijdenbos, A., Worsley, K., Collins, D. L., Blumenthal, J., Giedd, J. N., et al. (1999). Structural maturation of neural pathways in children and adolescents: In vivo study. *Science, 283*, 1908–1911.

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence, 17*(2).

Petrides, M., Alivisatos, B., Evans, A. C., & Meyer, E. (1993). Dissociation of human mid-dorsolateral from posterior dorsolateral frontal cortex in memory processing. *Proceedings of the National Academy of Sciences, 90*, 873-877.

Petridou, N., Schäfer, A., Gowland, P., & Bowtell, R. (2009). Phase vs. magnitude information in functional magnetic resonance imaging time series: toward understanding the noise. *Magnetic Resonance Imaging, 27*(8), 1046-1057.

Pfefferbaum, A., Adalsteinsson, E., & Sullivan, E. V. (2006). Supratentorial profile of white matter microstructural integrity in recovering alcoholic men and women. *Biological Psychiatry, 59*(4), 364-372.

Pfefferbaum, A., Desmond, J. E., Galloway, C., Menon, V., Glover, G. H., & Sullivan, E. V. (2001). Reorganization of frontal systems used by alcoholics for spatial working memory: an fMRI study. *NeuroImage, 14*(1 Pt 1), 7-20.

Pfefferbaum, A., Mathalon, D. H., Sullivan, E. V., Rawles, J. M., Zipursky, R. B., & Lim, K. O. (1994). A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. *Archives of Neurology, 51*(9), 874-887.

Pfefferbaum, A., Rosenbloom, M., Rohlfing, T., & Sullivan, E. V. (2009). Degradation of association and projection white matter systems in alcoholism detected with quantitative fiber tracking. *Biological Psychiatry, 65*(8), 680-690.

Pfefferbaum, A., & Sullivan, E. V. (2005). Disruption of brain white matter microstructure by excessive intracellular and extracellular fluid in alcoholism: Evidence from diffusion tensor imaging. *Neuropsychopharmacology, 30*, 423-432.

Pfefferbaum, A., Sullivan, E. V., Hedehus, M., Adalsteinsson, E., Lim, K. O., & Moseley, M. (2000). In vivo detection and functional correlates of white matter microstructural disruption in chronic alcoholism. *Alcoholism: Clinical & Experimental Research, 24*(8), 1214-1221.

Pfefferbaum, A., Sullivan, E. V., Mathalon, D. H., & Lim, K. O. (1997). Frontal lobe volume loss observed with magnetic resonance imaging in older chronic alcoholics. *Alcoholism: Clinical & Experimental Research, 21*, 521–529.

Pfefferbaum, A., Sullivan, E. V., Rosenbloom, M. J., Mathalon, D. H., & Lim, K. O. (1998). A controlled study of cortical gray matter and ventricular changes in alcoholic men over a 5-year interval. *Archives of General Psychiatry, 55*(10), 905-912.

Postle, B. R., & D'Esposito, M. (1999). "What"-Then-Where" in visual working memory: An event-related fMRI study. *Journal of Cognitive Neuroscience, 11*(6), 585-597.

Pulido, C., Anderson, K. G., Armstead, A. G., Brown, S. A., & Tapert, S. F. (2009). Family history of alcohol-use disorders and spatial working memory: effects on adolescent alcohol expectancies. *Journal of Studies on Alcohol & Drugs, 70*(1), 87-91.

R Development Core Team. (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, V., Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rensink, R. A. (2000a). The dynamic representation of scenes. *Visual Cognition, 7*, 17-42.

Rensink, R. A. (2000b). Visual search for change: A probe into the nature of attentional processing. *Visual Cognition, 7*, 345-376.

Rensink, R. A. (2002). Change detection. *Annual Review of Psychology, 53*, 245-277.

Rey, A., & Osterrieth, P. A. (1993a). Translations of excerpts from Andre Rey's "Psychological examination of traumatic encephalopathy" and P.A. Osterreich's "The complex figure copy test" (J. Corwin & F. W. Bylsma, Trans.). *The Clinical Neuropsychologist, 7*, 3-21.

Rey, A., & Osterrieth, P. A. (1993b). Translations of excerpts from Andre Rey's "Psychological examination of traumatic encephalopathy" and P.A. Osterrieth's "The complex figure copy test" (J. Corwin & F. W. Bylsma, Trans.). *The Clinical Neuropsychologist, 7*, 3-21.

Rice, J. P., Reich, T., Bucholz, K. K., Neuman, R. J., Fishman, R., Rochberg, N., et al. (1995). Comparison of direct interview and family history diagnoses of alcohol dependence. *Alcoholism: Clinical and Experimental Research, 19*, 1018–1023.

Robins, L., Cotter, L., Bucholz, K., & Compton, W. (1996). The Diagnostic Interview Schedule, Version 4.0 (DIS 4.0). *St. Louis, MO: Washington University School of Medicine*.

SAMHSA. (2008). *Substance Abuse and Mental Health Services Administration, Office of Applied Studies (2008). Results from the 2007 National Survey on Drug Use and Health: National Findings (NSDUH Series H-34, DHHS Publication No. SMA 08-4343).* Rockville, MD.

Schmithorst, V. J., Wilke, M., Dardzinski, B. J., & Holland, S. K. (2005). Cognitive functions correlate with white matter architecture in a normal pediatric population: a diffusion tensor MRI study. *Human Brain Mapping, 26*(2), 139–147.

Schweinsburg, A. D., McQueeny, T., Nagel, B. J., Eyler, L. T., & Tapert, S. F. (2010). A preliminary study of fMRI response during verbal encoding among adolescent binge drinkers. *Alcohol, 44*(1), 111-117.

Schweinsburg, A. D., Nagel, B. J., & Tapert, S. F. (2005). fMRI reveals alteration of spatial working memory networks across adolescence. *Journal of the International Neuropsychological Society, 11*(5), 631-644.

Schweinsburg, A. D., Schweinsburg, B. C., Cheung, E. H., Brown, G. G., Brown, S. A., & Tapert, S. F. (2005). fMRI response to spatial working memory in adolescents with comorbid marijuana and alcohol use disorders. *Drug & Alcohol Dependence, 79*, 201-210.

Schweinsburg, A. D., Schweinsburg, B. C., Nagel, B. J., Eyler, L. T., & Tapert, S. F. (2011). Neural correlates of verbal learning in adolescent alcohol and marijuana users. *Addiction, 106*(3), 564-573.

Shaffer, D., Fisher, P., Lucas, C. P., Dulcan, M. K., & Schwab-Stone, M. E. (2000). NIMH Diagnostic Interview Schedule for Children Version IV (NIMH DISC-IV): Description, differences from previous versions, and reliability of some common diagnoses. *Journal of the American Academy of Child and Adolescent Psychiatry, 39*, 28–38.

Sher, K. J., Martin, E. D., Wood, P. K., & Rutledge, P. C. (1997). Alcohol use disorders and neuropsychological functioning in first-year undergraduates. *Experimental and Clinical Psychopharmacology, 5*(3), 304-315.

Slutske, W. S., Thomas M. Piasecki, T. M., & Hunt-Carter, E. E. (2003). Development and Initial Validation of the Hangover Symptoms Scale: Prevalence and Correlates of Hangover Symptoms in College Students. *Alcoholism: Clinical & Experimental Research, 27*(9), 1442–1450.

Smith, E. E., & Jonides, J. (1999). Storage and executive processes in the frontal lobes. *Science, 283*, 1657-1661.

Sobell, L. C., & Sobell, M. B. (1992). Timeline Follow-back: A technique for assessing self-reported ethanol consumption. In J. Allen & R. Z. Litten (Eds.), *Measuring Alcohol Consumption: Psychosocial and Biological Methods* (pp. 41-72). Totowa, NJ: Humana Press.

Sowell, E. R., Thompson, P. M., Holmes, C. J., Jernigan, T. L., & Toga, A. W. (1999). In

vivo evidence for post adolescent brain maturation in frontal and striatal regions. *Nature Neuroscience, 2*(10), 859-861.

Sowell, E. R., Thompson, P. M., M., L. C., Welcome, S. E., Kan, E., & Toga, A. W. (2004). Longitudinal mapping of cortical thickness and brain growth in normal children. *Journal of Neuroscience, 24*(38), 8223-8231.

Sowell, E. R., Thompson, P. M., Tessner, K., & Toga, A. W. (2001). Mapping continued brain growth and gray matter density reduction in dorsal frontal cortex: Inverse relationships during postadolescent brain maturation. *Journal of Neuroscience, 21*, 8819–8829.

Spadoni, A. D., Norman, A. L., Schweinsburg, A. D., & Tapert, S. F. (2008). Effects of family history of alcohol use disorders on spatial working memory BOLD response in adolescents. *Alcoholism: Clinical and Experimental Research, 32*(7), 1135-1145.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience & Biobehavioral Reviews, 24*(4), 417-463.

Spear, L. P. (2002). The adolescent brain and the college drinker: Biological basis of propensity to use and misuse alcohol. *Journal of Studies on Alcohol, Suppl. 14*, 71-81.

Spear, L. P., & Varlinskaya, E. I. (2005). Adolescence. Alcohol sensitivity, tolerance, and intake. *Recent Developments in Alcoholism, 17*, 143-159.

Specht, K., Willmes, K., Shah, N., & Jancke, L. (2003). Assessment of reliability in functional imaging studies. *Journal of Magnetic Resonance Imaging, 17*, 463-471.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

SPSS for Windows. Rel. 18.0.0. 2009. Chicago: SPSS Inc.

Squeglia, L. M., Dager Schweinsburg, A., Pulido, C., & Tapert, S. F. (2011). Adolescent binge drinking linked to abnormal spatial working memory brain activation: differential gender effects. *Alcoholism: Clinical and Experimental Research,* 35 (10), 1831-41.

Squeglia, L. M., Jacobus, J., & Tapert, S. F. (2009). The influence of substance use on adolescent brain development. *Journal of Clinical EEG & Neuroscience, 40*(1), 31-38.

Squeglia, L. M., Pulido, C., Spadoni, A. D., Infante, M. A., & Tapert, S. F. (June, 2009). Heavy drinking adolescents show reorganized activation patterns during visual working memory. Poster presented at the Research Society on Alcoholism, San Diego, CA.

Squeglia, L. M., Sorg, S. F., Dager Schweinsburg A., Wetherill, R. R., Pulido, C. & Tapert,

S. F. (2012). Binge drinking differentially affects adolescent male and female brain morphometry. *Psychopharmacology*, 220 (3), 529-39.

Squeglia, L. M., Spadoni, A. D., Infante, M. A., Myers, M. G., & Tapert, S. F. (2009). Initiating moderate to heavy alcohol use predicts changes in neuropsychological functioning for adolescent girls and boys. *Psychology of Addictive Behaviors, 23*(4), 715-722.

Steer, R. A., Geetha, K., Ranieri, W. F., & Beck, A. T. (1998). Use of the Beck Depression Inventory-II with adolescent psychiatric outpatients. *Journal of Psychopathology and Behavioral Assessment, 20*, 127-137.

Stefanovic, B., Warnking, J. M., Rylander, K. M., & Pike, G. B. (2006). The effect of global cerebral vasodilation on focal activation hemodynamics. *Neuroimage, 30*(3), 726-734.

Stevens, G., & Featherman, D. L. (1981). A revised socioeconomic index of occupational status. *Social Science Research, 10*, 364-395.

Sullivan, E. V., & Pfefferbaum, A. (2005). Neurocircuitry in alcoholism: a substrate of disruption and repair. *Psychopharmacology, 180*(4), 583-594.

Suzuki, Y., Oishi, M., Mizutani, T., & Sato, Y. (2002). Regional cerebral blood flow measured by the resting and vascular reserve (RVR) method in chronic alcoholics. *Alcoholism: Clinical & Experimental Research, 26*(8), 95S-99S.

Talairach, J., & Tournoux, P. (1988). *Coplanar stereotaxic atlas of the human brain. Three-dimensional proportional system: An approach to cerebral imaging.* New York: Thieme.

Tapert, S. F., Brown, G. G., Kindermann, S. S., Cheung, E. H., Frank, L. R., & Brown, S. A. (2001). fMRI measurement of brain dysfunction in alcohol-dependent young women. *Alcoholism: Clinical & Experimental Research, 25*(2), 236-245.

Tapert, S. F., & Brown, S. A. (1999). Neuropsychological correlates of adolescent substance abuse: Four-year outcomes. *Journal of the International Neuropsychological Society, 5*, 481-493.

Tapert, S. F., & Brown, S. A. (2000). Substance dependence, family history of alcohol dependence and neuropsychological functioning in adolescence. *Addiction, 95*(7), 1043-1053.

Tapert, S. F., Granholm, E., Leedy, N. G., & Brown, S. A. (2002). Substance use and withdrawal: Neuropsychological functioning over 8 years in youth. *Journal of the International Neuropsychological Society, 8*, 873-883.

Tapert, S. F., Pulido, C., Paulus, M. P., Schuckit, M. A., & Burke, C. (2004). Level of

response to alcohol and brain response during visual working memory. *Journal of Studies on Alcohol, 65*(6), 692-700.

Tapert, S. F., Schweinsburg, A. D., Barlett, V. C., Brown, S. A., Frank, L. R., Brown, G. G., et al. (2004). Blood oxygen level dependent response and spatial working memory in adolescents with alcohol use disorders. *Alcoholism: Clinical & Experimental Research, 28*(10), 1577-1586.

Tapert, S. F., Schweinsburg, A. D., Drummond, S. P. A., Paulus, M. P., Brown, S. A., Yang, T. T., et al. (2007). Functional MRI of inhibitory processing in abstinent adolescent marijuana users. *Psychopharmacology, 194*, 173-184.

Tapert, S. F., Theilmann, R. J., & Schweinsburg, A. D. (2003). Reduced fractional anisotropy in the splenium of adolescents with alcohol use disorder. *Proceedings of the International Society of Magnetic Resonance Medicine, 11*(8217).

Tarter, R. E., Mezzich, A. C., Hsieh, Y.-C., & Parks, S. M. (1995). Cognitive capacity in female adolescent substance abusers. *Drug and Alcohol Dependence, 39*, 15-21.

Thoma, R. J., Monnig, M. A., Lysne, P. A., Ruhl, D. A., Pommy, J. A., Bogenschutz, M., et al. (2011). Adolescent substance abuse: the effects of alcohol and marijuana on neuropsychological performance. *Alcoholism: Clinical & Experimental Research, 35*(1), 39-46.

Tjandra, T., Brooks, J. C., Figueiredo, P., Wise, R., Matthews, P. M., & Tracey, I. (2005). Quantitative assessment of the reproducibility of functional activation measured with BOLD and MR perfusion imaging: implications for clinical trial design. *NeuroImage, 27*, 393-401.

Tomasi, D. G., & Caparelli, E. C. (2007). Macrovascular contribution in activation patterns of working memory. *Journal of Cerebral Blow Flow & Metabolism, 27*, 33-42.

Tuch, D. S., Salat, D. H., Wisco, J. J., Zaleta, A. K., Hevelone, N. D., & Rosas, H. D. (2005). Choice reaction time performance correlates with diffusion anisotropy in white matter pathways supporting visuospatial attention. *Proceedings of the National Academy of Sciences, 102*(34), 12212-12217.

Twitchell, G., Hertzog, C., Klein, J., & Schuckit, M. (1992). The anatomy of a follow-up. *Addiction, 87*(9), 1327-1333.

Ungerleider, L. G., Courtney, S. M., & Haxby, J. V. (1998). A neural system for human visual working memory. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 883-890.

Vik, P. W., Cellucci, T., Jarchow, A., & Hedt, J. (2004). Cognitive impairment in substance

abuse. *Psychiatric Clinics of North America, 27*(97-109).

Ward, B. D. (2000). Simultaneous inference for fMRI data. *Retrieved March 23, 2009, from http://afni.nimh.nih.gov/afni/doc/manual/AlphaSim*.

Wechsler, D. (1991). *Wechsler Intelligence Scale for Children (3rd ed.)*. New York: Psychological Corporation.

Wechsler, D. (1997). *Wechsler Adult Intelligence Scale (3rd ed.)*. San Antonio, TX: The Psychological Corporation.

Winters, K. C., Stinchfield, R. D., Henly, G. A., & Schwartz, R. (1990-91). Validity of adolescent self-report of alcohol and other drug involvement. *International Journal of the Addictions, 25*(11A), 1379-1395.

Yakovlev, P. I., & Lecours, A. R. (1967). The myelogenetic cycles of regional maturation of the brain. In A. Minkowski (Ed.), *Regional Development of the Brain in Early Life* (pp. 3-70). Boston: Blackwell Scientific.

Yeh, P. H., Simpson, K., Durazzo, T. C., Gazdzinski, S., & Meyerhoff, D. J. (2009). Tract-Based Spatial Statistics (TBSS) of diffusion tensor imaging data in alcohol dependence: Abnormalities of the motivational neurocircuitry. *Psychiatry Research: Neuroimaging, 173*(1), 22-30.

Zandbelt, B. B., Gladwin, T. E., Raemaekers, M., van Buuren, M., Neggers, S. F., Kahn, R. S., et al. (2008). Within-subject variation in BOLD-fMRI signal changes across repeated measurements: quantification and implications for sample size. *NeuroImage, 42*(1), 196-206.

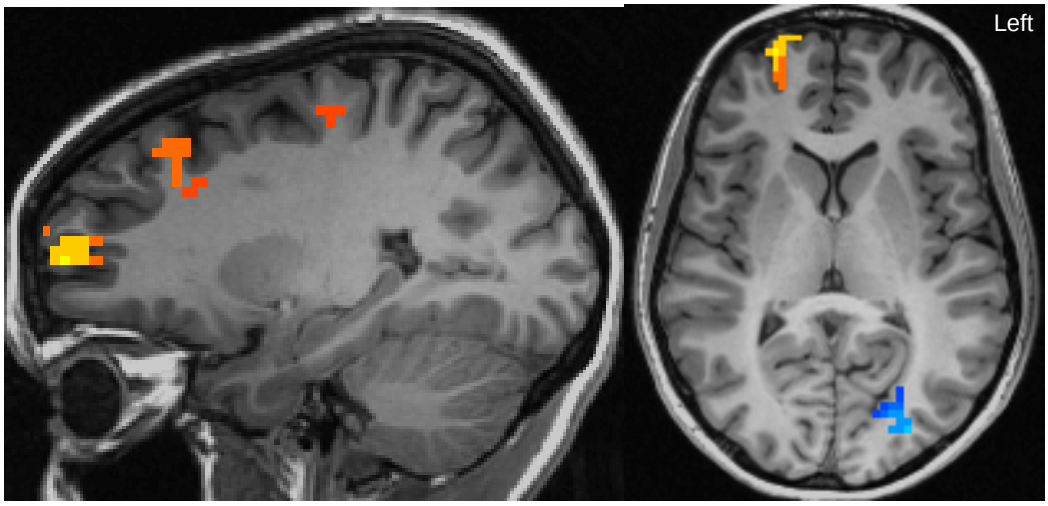


Figure 1. Preliminary cross-sectional analyses (Squeglia et al., June, 2009) used to define regions of interest for hypotheses. Warm colors show where Heavy Drinkers (*n*=20) had significantly (corrected $p<.05$, clusters $\geq$ 908μL) more BOLD response contrast than Controls (*n*=20) for high relative to low VWM load (i.e., right inferior parietal, right middle frontal, right superior frontal, and bilateral medial frontal regions). Cool colors show where Heavy Drinkers had significantly less response than Controls (i.e., left middle occipital and bilateral anterior cingulate cortex).

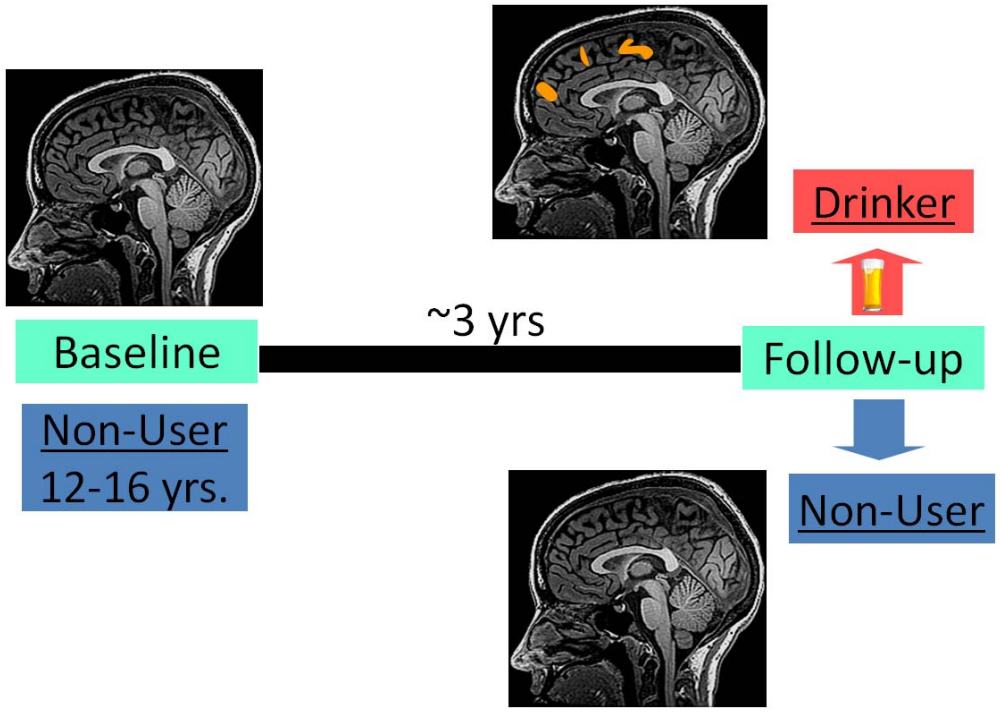


Figure 2. Model: Adolescent heavy drinking impairs neurocognitive functioning over time. Participants were matched 1:1 on baseline and follow-up age, pubertal development, years since baseline, gender, and family history of AUD.

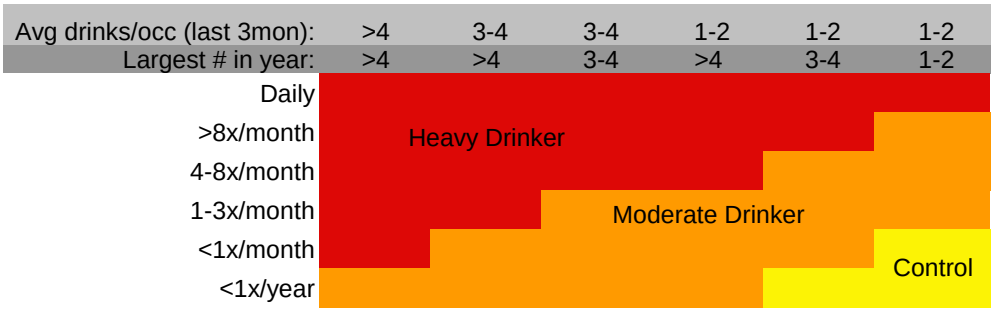


Figure 3. Outcome drinking classification, based on Cahalan et al., 1969 (Cahalan, Cisin, & Crossley, 1969) and modified based on the distribution of drinking characteristics of adolescent males and females observed in the first two years of this project (Schweinsburg et al., 2005; Tapert et al., 2004).

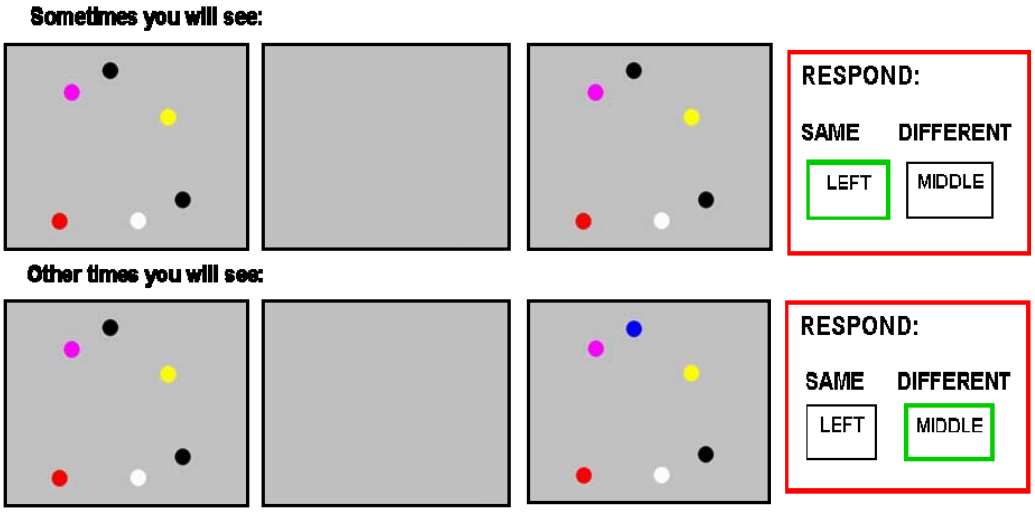


Figure 4: Visual Working Memory task (Tapert, Pulido, et al., 2004). The 6-dot (high working memory load) vs. 2 dot (low working memory load) BOLD activation difference was used as dependent variable in analyses.

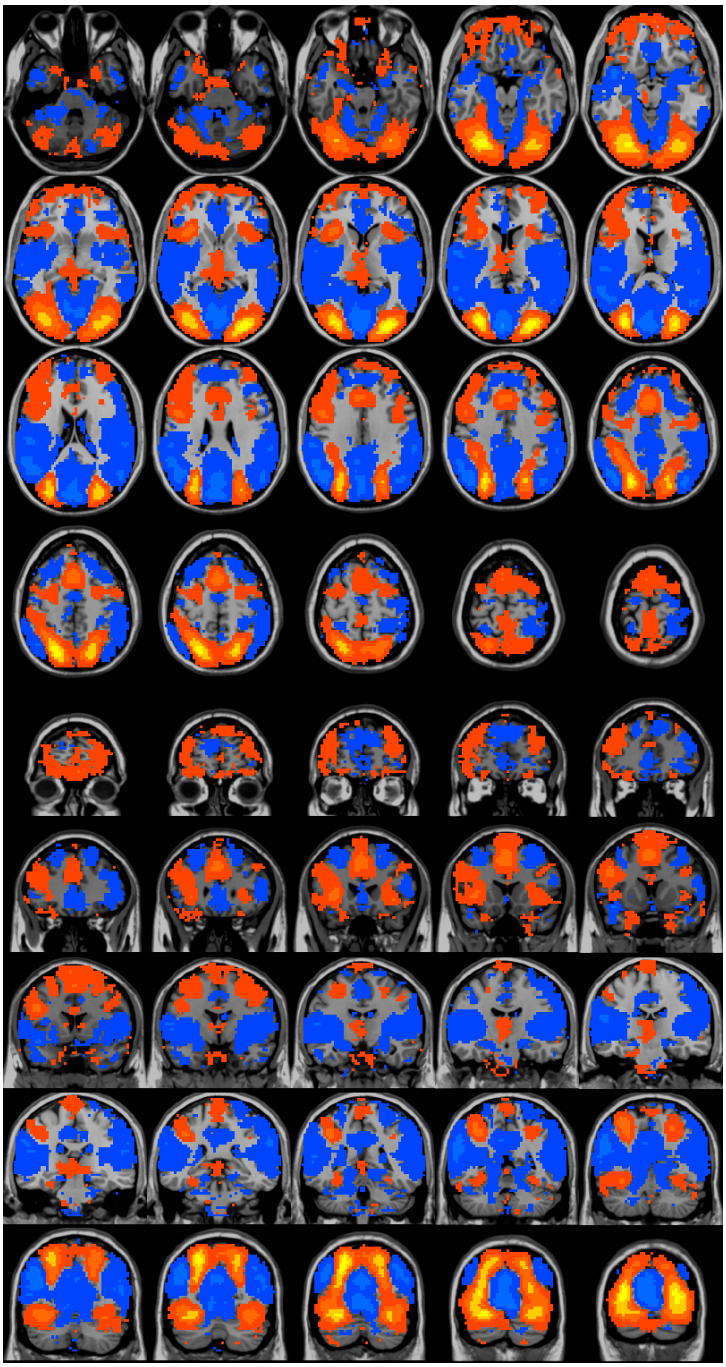


Figure 5: Whole brain unthresholded BOLD activation to the 6 vs. 2 dot task (*N*=80). Axial and coronal views. Orange/yellow colors indicate positive BOLD response (greater 6 vs. 2 dot activation), while blue areas indicate negative BOLD response (i.e., "deactivation"; greater 2 vs. 6 dot activation).

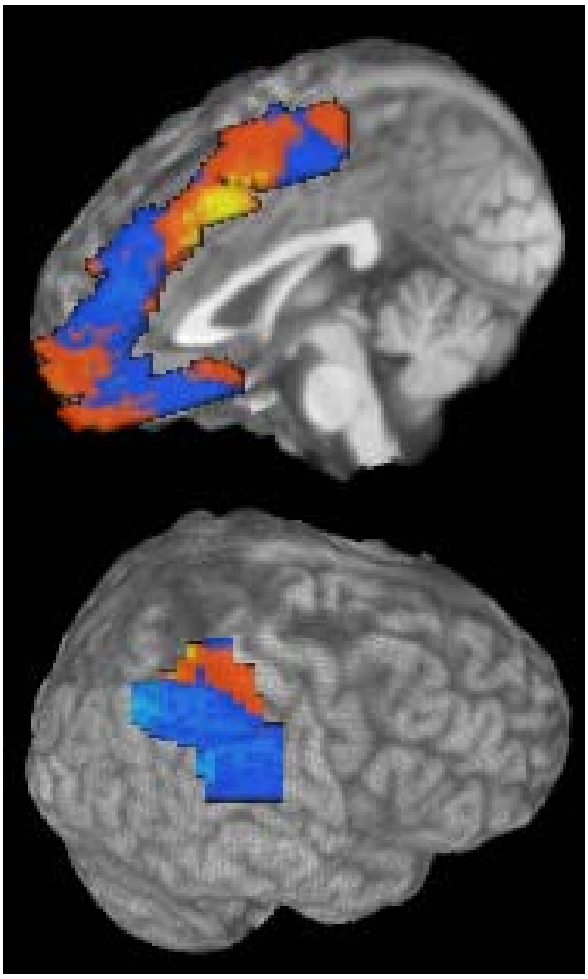


Figure 6: Region of interest (i.e., left medial frontal gyrus and right inferior parietal lobule) BOLD activation to the 6 vs. 2 dot task (*N*=80). Orange/yellow colors indicate positive BOLD response (greater 6 vs. 2 dot activation), while blue areas indicate negative BOLD response (i.e., "deactivation"; greater 2 vs. 6 dot activation).

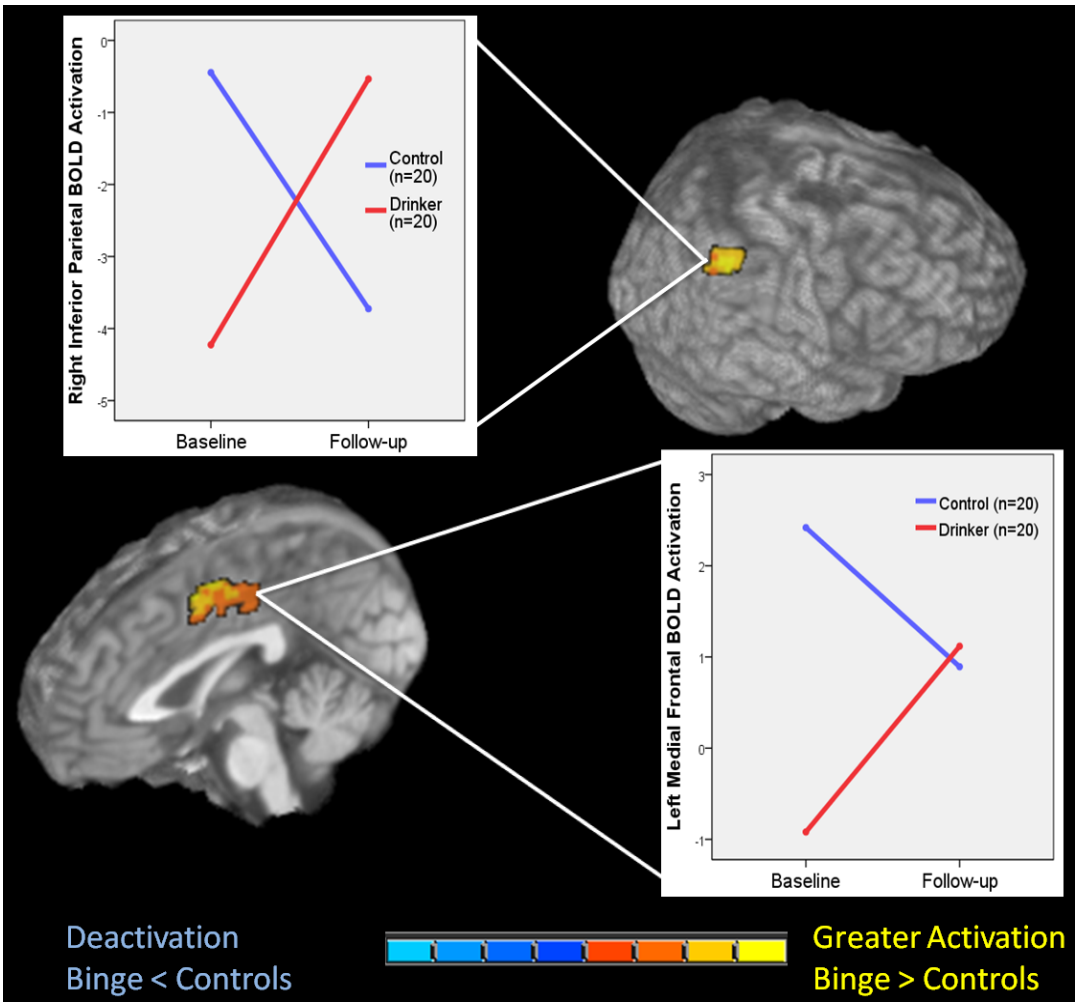
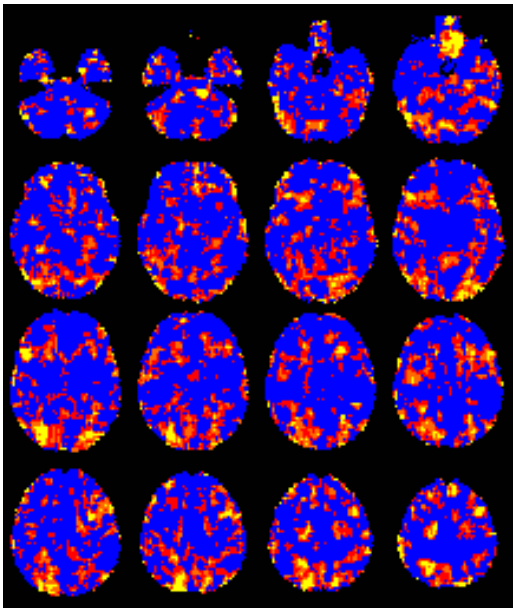


Figure 7: Significant group x time interactions were found in two of the five hypothesized regions of interest, including right inferior parietal lobule (cluster size: 810µL, *p*=.005) and left medial frontal gyrus (cluster size: 1431µL, *p*=.003). At baseline, significant differences in BOLD activation were observed in both regions. At follow-up, differences in BOLD activation were trending towards significant (*p*=.10) in the right inferior parietal lobule. While controls did not change significantly over time, heavy drinkers did in both regions.

Baseline:



Follow-up:

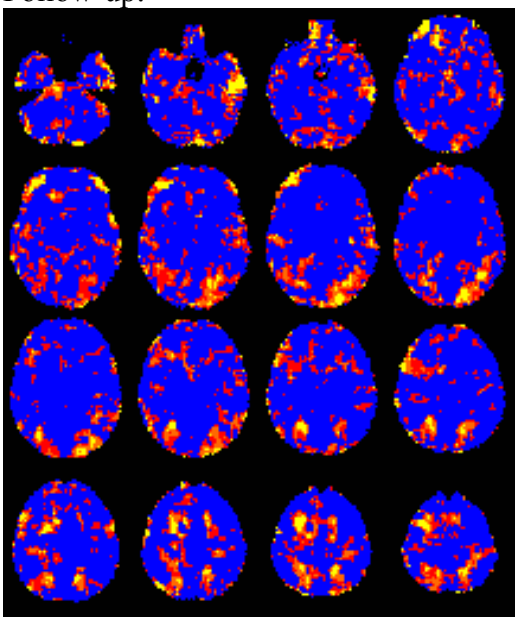


Figure 8: Within-session intraclass correlations for baseline and follow-up time points on separate sample of non-using adolescents (N=24; 48 total scans). Color scheme using Cicchetti and Sparrow's 1981 criteria for reliability: blue=poor: <0.40; red=fair: 0.40–0.59; orange=good: 0.60–0.74; yellow=excellent: >0.74 reliability.

Table 1. Demographic characteristics at baseline and follow-up.

	Continuous Controls (*n*=20)	**Heavy Drinking Transitioners (*n*=20)**
Baseline		
Age	14.77 (1.14)	15.07 (1.26)
Gender (% males)	70%	70%
Race (% Caucasian) [b]	55%	65%
Family history of alcoholism density (range 0-2)	0.19 (0.31)	0.40 (0.63)
Conduct Disorder positive (%)	0%	0%
Hollingshead Index of Social Position score	27.40 (16.30)	19.10 (12.08)
Parent salary ($) [a]	$109K (63K)	$171K (103K)
Years of education	8.10 (1.29)	8.75 (1.37)
Female Pubertal Development Scale total	15.83 (2.48)	18.00 (2.10)
Male Pubertal Development Scale total	13.79 (2.75)	14.15 (3.16)
Beck Depression Inventory total	2.20 (2.61)	1.89 (2.62)
Spielberger State Anxiety total	26.15 (6.04)	26.94 (5.91)
CBCL/YSR internalizing *T*-score	46.47 (9.28)	44.25 (10.36)
CBCL/YSR externalizing *T*-score	39.76 (6.51)	42.81 (8.38)
Sleepiness rating before scan	4.60 (1.47)	4.68 (1.83)
Sleepiness rating after scan	5.70 (1.78)	5.89 (2.13)
GPA	3.35 (.74)	3.53 (.45)
Follow-up		
Age	17.71 (1.44)	18.46 (1.90)
Years between scans	2.94 (0.98)	3.43 (1.07)
Conduct Disorder positive (%)	0%	15%
Female Pubertal Development Scale total	19.43 (0.98)	20.00 (0.00)
Male Pubertal Development Scale total	17.33 (2.35)	17.29 (3.15)
Years of education	11.00 (1.41)	11.89 (2.11)
Beck Depression Inventory total	1.35 (1.84)	2.60 (3.33)
Spielberger State Anxiety total	25.00 (5.01)	22.68 (2.98)
CBCL/YSR internalizing *T*-score	40.37 (7.43)	41.58 (9.47)
CBCL/YSR externalizing *T*-score	41.74 (8.81)	46.32 (10.15)
Sleepiness rating before scan	3.90 (1.21)	4.25 (1.65)
Sleepiness rating after scan	5.60 (1.76)	6.15 (1.76)
GPA	3.48 (.39)	3.35 (.51)

[a] Continuous controls ≠ heavy drinkers, $p<.05$

[b] For the full sample, ethnicity was: 30% Latino; race was: 60% Caucasian, 35% multiracial, 2.5% African-American, 2.5% Asian.

Note: Significant time x drinking status interactions ($p<.05$) were observed for conduct disorder diagnosis.

Abbreviations: CBCL, Child Behavior Checklist; YSR, Youth Self Report.

Table 2. Substance use characteristics at baseline and follow-up.

	Continuous Controls (*n*=20)	**Heavy Drinking Transitioners (*n*=20)**
Baseline		
Lifetime alcohol use occasions [a]	0.05 (0.22)	1.50 (3.02)
Lifetime marijuana use occasions	0.00 (0.00)	0.10 (0.31)
Lifetime other drug use occasions	0.00 (0.00)	0.00 (0.00)
Follow-up		
Lifetime alcohol use occasions [a]	1.35 (1.90)	93.50 (79.56)
Peak drinks on an occasion, past year [a]	0.60 (0.94)	11.90 (5.61)
Peak drinks on an occasion, past 3 months [a]	0.25 (0.44)	8.80 (6.01)
Estimated peak BAC, past 3 months [a]	0.00 (0.01)	0.25 (0.14)
Average # drinks per drinking day, past month [a]	0.25 (0.55)	6.10 (4.28)
Days since last alcohol use	N/A	37.74 (70.75)
Tobacco cigarettes per day, past month	0.00 (0.00)	0.20 (0.62)
Lifetime marijuana use occasions [a]	0.15 (0.49)	83.55 (171.81)
Marijuana use days/month, past 3 months [a]	0.00 (0.00)	3.75 (6.33)
Lifetime other drug use occasions	0.00 (0.00)	1.50 (3.55)

[a] Continuous controls ≠ heavy drinkers, $p<.05$

Note: As expected, significant time x drinking status interactions were observed for lifetime alcohol, marijuana, and other drug use.

Table 3. Neuropsychological test scores at baseline and follow-up.

	Continuous Controls (*n*=20)	**Heavy Drinking Transitioners (*n*=20)**
Baseline		
Complex Figure copy accuracy	29.93 (3.27)	29.53 (2.49)
Complex Figure delay accuracy	20.55 (6.07)	20.50 (5.06)
WASI Block Design	46.75 (12.99)	52.85 (12.25)
WAIS-III Digits forward [a]	8.95 (1.96)	10.35 (2.25)
WAIS-III Digits backward	5.60 (1.64)	6.85 (2.74)
DVT completion time (seconds)	209.05 (48.43)	199.11 (48.49)
WRAT3 Reading scaled score	107.50 (11.33)	110.25 (6.90)
Follow-up		
Complex Figure copy accuracy	29.84 (3.37)	29.55 (2.53)
Complex Figure delay accuracy	21.66 (4.37)	21.05 (5.34)
WASI Block Design	56.15 (11.39)	58.75 (8.29)
WAIS-III Digits forward [a]	9.65 (1.95)	11.40 (2.74)
WAIS-III Digits backward [a]	6.80 (1.74)	8.50 (3.22)
DVT completion time (seconds) [a]	189.85 (36.29)	167.68 (30.23)
WRAT3 Reading scaled score	105.80 (11.81)	110.05 (11.00)

[a] Continuous controls ≠ heavy drinkers, $p<.05$

Note: No significant time x drinking status interactions ($p<.05$) were observed for neuropsychological test variables.

Table 4. fMRI task performance data at baseline and follow-up.

	Continuous Controls (*n*=20)	**Heavy Drinking Transitioners (*n*=20)**
Baseline		
2-dot accuracy (%)	91.01 (8.24)	91.18 (11.27)
6-dot accuracy (%)	77.93 (12.24)	79.40 (8.07)
2-dot reaction time (ms) [a]	2596.89 (217.96)	2436.22 (223.73)
6-dot reaction time (ms)	2714.00 (230.55)	2599.56 (214.30)
Follow-up		
2-dot accuracy (%)	96.11 (4.50)	94.86 (4.95)
6-dot accuracy (%)	84.04 (8.35)	80.16 (10.27)
2-dot reaction time (ms)	2213.89 (125.95)	2214.10 (96.40)
6-dot reaction time (ms)	2369.32 (147.57)	2405.50 (104.57)

[a] Continuous controls ≠ heavy drinkers, $p<.05$

Note: Significant time x drinking status interactions were observed in 2- and 6-dot reaction time. For both interactions, adolescents who transitioned into heavy drinking had attenuated decreases in reaction time at follow-up compared to continuous controls.

Table 5. Significant drinking status by time interactions for BOLD response to visual working memory (N=40).

Brain region	BA	Volume (μl)	Talairach Coordinates [a]			Peak Activation *M* (SD)				η^2
			x	y	z	BL Controls	BL Future Drinkers	FU Controls	FU Heavy Drinkers	
R inferior parietal lobule	40, 13	810	-52.5	46.5	38.5	-0.45 (3.90)	-4.23 (5.11)	-3.73 (6.10)	-0.54 (6.30)	0.23
L medial frontal gyrus	6	1431	4.5	-1.5	53.5	2.42 (2.95)	-0.92 (3.17)	0.89 (2.22)	1.12 (2.47)	0.19

R *right*; L *left;* BA=Brodmann Area(s)

[a] Coordinates refer to location of peak group difference in VWM response within the cluster.