Impact of regular low-level alcohol consumption on cognitive interference and response

inhibition:

An fMRI investigation in young adults

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Abstract

The purpose of the present dissertation was to shed light on the neurophysiological effect of regular consumption of low amounts of alcohol on two important aspects of executive functions, cognitive interference and response inhibition, using functional magnetic resonance imaging (fMRI) in a sample of young adults. Participants were recruited from the Ottawa Prenatal Prospective Study (OPPS), a longitudinal study that has collected data from participants from infancy to young adulthood, which permitted control of a number of potentially confounding drug and lifestyle variables. This allowed for investigation of the unique effect of alcohol use on executive functions. The dissertation itself is comprised of two original manuscripts: the first study compared low-level alcohol users to controls on performance of the Counting Stroop, a task of cognitive interference; and the second study compared users to controls on performance of the Go/No-Go, a task of response inhibition.

Although the results of both studies found no performance differences between groups, low-level alcohol users had significantly more brain activation in several regions, including areas not typically associated with task processing, compared to irregular or non-drinker controls. This difference in neurophysiology may be reflective of compensatory strategies within the brain, whereby the recruitment of additional regions may be attempting to compensate for potential underlying deficits that occur with increasing cognitive demand. While further research is needed to validate this hypothesis, the present findings highlight the vulnerability of the developing brain.

Statement of co-authorship

The two original manuscripts included in this dissertation were prepared in collaboration with my dissertation supervisor, Dr. Andra M. Smith, who provided support and guidance on all aspects of the dissertation. Dr. Peter A. Fried was included as a co-author, as study participants were recruited from his longitudinal research sample. Dr. Ian Cameron and Dr. Matthew J. Hogan were also included as co-authors for their assistance on MRI settings (e.g. physics of pulse sequence of MRI scanning) during original data collection. Rebecca E. Halchuk and Carmelinda A. Longo were included as co-authors because of their assistance during the original data collection process. Ola Mioduszewski and Carley Fall were included as co-authors for their assistance in data cleaning and pre-processing, and lastly Aziza Byron-Alhassan was included as a co-author for her assistance in editing manuscripts. As the first author of both manuscripts, I was primarily responsible for the conceptualization of research hypotheses, methods, planning and completing all statistical analyses, and all manuscript writing and preparation.

Acknowledgements

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Chapter 1

General Introduction

Alcohol use in Canada

In 2017, alcohol continues to be the most commonly used drug in Canada across the population and is often the first substance used among Canadian youth. For instance, according to the Canadian Alcohol and Drug Use Monitoring Survey (CADUMS), more than 78.4% of the general population reported consuming alcohol over the past year. This includes 70% of youth (ages 15-24) and 80% of adults (ages 25 and older), with an average age of initial drinking onset of 16.2 years old. However, the majority of adults consuming one to two servings of alcohol per day (i.e. a four-ounce glass of wine, a 12-ounce can of beer, of a 1.5-ounce shot of distilled spirit) will not experience any detrimental side effects. In fact, some research suggests possible health benefits from regularly consuming small amounts of alcohol, such as decreasing the risk of cardiovascular disease and lowering the risk of Alzheimer's disease (Arntzen et al., 2010; Hines and Rimm, 2001; Sayed & French, 2016).

Despite these potential benefits, heavy alcohol use has a significant harmful effect on overall health. For example, alcohol abuse is associated with liver damage, increasing the risk of cirrhosis. Liver cancer is also one of the fastest rising types of cancer in Canada, increasing 2.3% per year for men and 2.4% for women, with alcohol abuse being associated with higher risk of occurrence, as well as being a key factor in increasing national prevalence rates (Canadian Cancer Statistics, 2015). Other potential negative consequences of alcohol abuse include increased risk of alcohol-related dementia, aerodigestive cancers, cardiomyopathy, haemorrhagic stroke, as well as acute issues that arise such as vehicular fatalities, violence, and suicide (Donovan et al., 2015; Hines and Rimm, 2001; Grune et al., 2017; Ridley, Draper, & Withall, 2013). According to the CADUMS, 3.6% of Canadians (an estimated 886,000 people) ages 15 years old and older reported regular alcohol abuse or dependency, making this a significant

health concern among our population. According to the literature, alcohol use patterns can be broken down into categories of low (1 to 7 drinks/week), moderate (8 to 14 drinks/week), and heavy (>15 drinks/week; Paul et al., 2008).

Impact of alcohol on brain and behaviour

The short-term impact of alcohol consumption is associated with a number of changes in cognition, such as lowered inhibitions (e.g. more talkative, friendly, flirtatious), and reduced attentional processing leading to impairments in judgment, decision-making and problem solving skills, and memory impairments (Dougherty, Marsh, Moeller, Chokshi, & Rosen, 2000; Grune et al., 2017; Jarosz, Colflesh, & Wiley, 2012; Oscar-Berman & Marinkovic, 2007). Moreover, through its impact on cognition, alcohol use can also lead to changes in emotional wellbeing. For example, Curtin et al. (2001) found that intoxication caused reduced attentional processing of threat related cues, which in turn, led to a significant reduction in fear response, such as anticipatory fear, and lower response inhibition. Acute alcohol intoxication may also cause increased emotional reactivity and aggressive behaviours through increased vulnerability to negative affect and rumination, with reductions in self-awareness and empathy (Giancola, Josephs, Parrott, & Duke, 2010).

The cognitive and behavioural changes that occur during alcohol intoxication are thought to be mediated by its effect on neural processes in the brain. At the cellular level, it is well documented that acute alcohol intoxication causes changes in neurochemistry within a number of neurotransmitter systems, including excitation of gamma-aminobutyric acid (GABA), dopamine, and serotonin, and inhibition of glutamate (Valenzuela, 1997; Volkow et al., 2008). For example, increased transmission of the inhibitory neurotransmitter GABA is thought to facilitate the anxiolytic effect of alcohol, which leads to the behavioural changes such as lowered inhibitions

and increased risk taking (Gilpin, Herman, & Roberto, 2015; Davies, 2003). Moreover, some research suggests that there is an inhibitory effect of alcohol on glutamate receptors and excitatory response at $GABA_A$ receptors within the hippocampus. This change in neural transmission may explain alcohol-related memory impairments (e.g. "blackouts") by preventing formation of long-term potentiation, a process fundamental for memory formation (Tokuda, Izumi, & Zorumski, 2013; Ziegler et al., 2005). Lastly, acute alcohol consumption also increases levels of dopamine and serotonin transmission, which may be responsible for its rewarding and reinforcing effect (Clapp, Bheave, & Hoffman, 2008).

Together, the changes in neural chemistry lead to widespread changes across the brain, including the prefrontal cortex (PFC), the limbic system, and the cerebellum (Oscar-Berman & Marinkovic, 2007). The PFC is an area of the brain considered to be responsible for higher order cognitive processes, executive functions, and regulation of its connections with other brain regions (Blair, 2016; Pokhrel et al., 2013; Siddiqui, Chatterjee, Kumar, Siddiqui, &Goyal, 2008). The limbic system works to regulate emotions, learning, and memory through the amygdala, hypothalamus, and hippocampus (Rajmohan & Mohandas, 2007). The cerebellum is the area of the brain primarily responsible for motor control and coordinating movement (Manto et al., 2012). Thus, many of the behavioural effects of alcohol intoxication, such as impaired decision-making, emotional reactivity, alcohol-related memory impairments, and clumsiness, are attributable to how it selectively impacts these brain regions.

In adulthood, prolonged alcohol abuse can lead to substantial changes in brain functioning, particularly in the white and grey matter of the PFC (Sullivan and Pfefferbaum, 2005). For example, Fortier et al. (2014) found that chronic alcohol use (average of 25 years of use) caused significant and widespread bilateral reductions in white matter integrity in a number

of regions, including the frontal, parietal, temporal, and cerebellar areas compared to non-

alcoholic controls. The most prominent loss occurred within the frontal and superior white matter

tracts, which included the superior and inferior frontal gyrus, precentral gyrus, and cingulate.

The amount of reduction in the inferior frontal gyrus also increased with heavier drinking.

Moreover, the long-term damage of the frontostriatal circuit from long-term alcohol abuse may

help explain difficulties with sobriety, as inhibitory control is controlled within this circuitry. In

addition, Paul et al. (2008) found a significant negative linear relationship between total cerebral

volume and alcohol consumption. More specifically, results indicated that as alcohol use

increased, cerebral volume decreased, highlighting that alcohol related structural brain damage

occurs along a dosage continuum.

Unsurprisingly, the PFC, parietal lobe, limbic system, and cerebellum have also been

identified as the brain structures most vulnerable to the negative effects of alcohol following

prolonged chronic use and abuse (Crews et al., 2005; Jacobus & Tapert, 2014). For example, De

Bellis et al. (2005) found that male youth (ages 13 to 21) who met criteria for alcohol use

disorder (AUD) had smaller overall volumes in the PFC compared to healthy controls, with

smaller volumes found in males than females with AUD. In contrast, Medina et al. (2008) found

structural abnormalities in the PFC of adolescents (age 15 to 17) with AUD compared to age

matched healthy controls. However, females with AUD showed smaller PFC volumes relative to

males with AUD. Nevertheless, these two studies highlight the vulnerability of the adolescent

PFC to developmental, and potentially long-lasting changes.

Nagel et al. (2005) investigated the impact of adolescent AUD on the hippocampus, an

important area of the limbic system responsible for new memory formation (Deng, Aimone, &

Gage, 2010). After controlling for potential comorbid mental health problems, results showed

adolescents with AUD had significantly smaller left hippocampal volumes compared to healthy

controls, which may lead to functional memory impairments later in life. In a similar study, De

Bellis et al. (2000) found significantly smaller volumes in both right and left hippocampus

among youth (ages 13 to 21) with AUD. Moreover, the age of onset was positively correlated

with total hippocampal volume, meaning that the younger the age of onset, the smaller the

hippocampal volumes. Results also indicated a negative correlation between hippocampal

volume and the duration of AUD, suggesting that shorter duration of AUD was associated with

larger hippocampal volumes.

In a more recent study, Heikkinen et al. (2016) conducted a longitudinal investigation of

alcohol consumption during adolescence, comparing heavy drinkers to light drinking controls.

Participants were followed over a 10-year period, completing self-report measures at various

time points and underwent neuroimaging at the final assessment. Grey matter volumes between

heavy drinkers and light drinkers were compared using Voxel-Based Morphometry (VBM).

Their findings showed that heavy drinkers had significantly reduced grey matter volumes in a

number of areas, including the bilateral anterior cingulate cortex, right orbiotofrontal and frontal

cortex, right superior temporal gyrus, and right insular cortex relative to light drinkers. These

results suggest that alcohol abuse during adolescence is associated with atypical development of

grey matter in a number of regions important in cognitive control and executive processes.

Neurocognitive development in adolescence

Given the early age of onset of alcohol use among Canadian youth, it is important to

consider how alcohol may be disruptive to significant developmental processes and increase

neural vulnerability to damage. During the transition from childhood to adolescence, the brain

continues to go through significant structural and functional developmental changes, well into

young adulthood. Maintaining healthy brain development during childhood and adolescence is essential to optimal neurocognitive functioning later in adulthood.

Among the developmental processes that occur during adolescence, there is a prominent reduction in the volume of grey matter that is attributable to the elimination of weak synaptic connections (Blakemore & Choudhury, 2006; Jacobus & Tapert, 2013). This pruning optimizes the brain for the challenges to come in young adulthood. Similarly, there are important changes that occur in white matter, as myelination continues to foster the development of fibre tracts that strengthen and increase speed of communication between different brain regions (Arain et al., 2013; Ladouceur, Peper, Crone, & Dahl, 2012). The prefrontal cortex (PFC) and the parietal cortex are two regions of the brain that have been consistently shown to undergo continued development during adolescence (Blakemore et al., 2006). In fact, research suggests that the PFC is the last cortical structure to reach maturity in the brain, which may continue to develop throughout the early 20s (Johnson, Blum, & Giedd, 2010; Rubia et al., 2000; Siddiqui et al., 2008).

Even small or subtle changes to typical neural development, including demyelination or changes in brain volume, during this critical period can have a long lasting functional impact in adulthood. This includes not only maladaptive changes to neural and cognitive function but also how these processes translate into important aspects of human behaviour, such as emotional and social functioning. This may have important implications for increasing vulnerability to adult psychopathology, emotion dysregulation, and risk-taking behaviours (Jacobus & Tapert, 2013). Thus, the developmental period of adolescence is a significant window of vulnerability where the brain is highly sensitive and susceptible to long-term changes that may arise from substance misuse. In terms of the role of alcohol in creating functional and structural changes, in remains

unclear whether chronic alcohol use plays a neurotoxic effect on brain development or whether it may cause abnormalities in developmental trajectories that ultimately prevent certain regions from reaching their full cortical volume or functional capacity (Heikkinen et al., 2016; Lopez-Larson et al., 2011). According to Silveri et al. (2016), the cortical areas most vulnerable to the negative impact of alcohol use during adolescence include the frontal lobe, followed by the temporal and parietal lobes. In addition to the structural abnormalities in the brain related to learning and memory processes that occur with adolescent drinking, many studies have reported that heavy alcohol use is associated with cognitive deficits, as well as poor academic and social performance (Guerri & Pascual, 2010).

Executive functions

Given the potential neurophysiological and behavioural implications of alcohol use among adolescent drinkers, it is particularly relevant to study how alcohol may impact important aspects of executive functioning. Executive functions refer to a set of interrelated higher-level cognitive processes responsible for the ability to self-regulate emotion, cognition, and behaviours (Blair, 2016; Karoly, 1993; Gyurak et al., 2009; Patrick, Blair, & Maggs, 2008). These processes are fundamental for goal-directed and future oriented behaviour, as well as regulating lower-order brain function, such as motor skills and sensory perception (Pokhrel et al., 2013; Spear, 2010; Welsh & Pennington, 1988). There are at least five fundamental executive function processes that have been identified, which include response inhibition, working memory, attention and interference control, cognitive flexibility, and planning and problem solving (Diamond, 2013; Duncan, McClelland, & Acock, 2017; Pokhrel et al., 2013; Welsh, Pennington, & Grossier, 1991).

These processes are called upon when automatic, reflexive responses or behaviours would be inadequate in maintaining or achieving goal-directed behaviours. Moreover, they are often interrelated and can work in an overlapping, collaborative way to achieve our goals (Diamond, 2013; Fuster, 2015; Pokhrel et al., 2013). Many domains of life, such as academics, work performance, as well as social and emotional functioning require skills such as organization, planning, and flexible thinking in order to achieve optimal performance. The neural processes underlying executive function are largely considered to occur within the PFC, with assistance from its connections with other cortical structures, depending on the type of processing (Blair, 2016; Pokhrel et al., 2013; Siddiqui et al., 2008). Given that these areas continue developing throughout adolescence, are vulnerable to alcohol related damage, and are important for higher order processes, examining the interaction between these processes warrants further investigation.

In summary, executive functions play a vital role in adaptive functioning across various domains in our day-to-day lives. As illustrated above, deficits in these processes can have a major impact on many important areas in life. Understanding how alcohol may fuel impairments in various domains of executive functioning, particularly through significant changes that could occur in the vulnerable, developing brain that may negatively alter functional trajectories in adulthood, is essential.

Neuroimaging

Magnetic resonance imaging (MRI) is a valuable method for exploring and understanding the pathology of alcohol use and its functional impact on neural processes. Functional MRI (fMRI) is a specific neuroimaging technique that permits researchers to observe how a given mental process is occurring in the brain. fMRI quantifies this activity in the brain by detecting

the changes that occur in blood flow and oxygenation in response to neural activation (Toma &

Nokai, 2002). When an area of the brain has increased neuronal activity, there are increases in

both oxygen consumption and blood flow in order to meet the increased energy demand

(Barinaga, 1997).

When neurons are inactive, oxygenated blood is converted to deoxygenated blood at a

constant rate within the capillaries of the brain. Oxygenated blood and deoxygenated blood have

different magnetic properties, with oxygenated blood being diamagnetic and deoxygenated blood

being paramagnetic. As a neuron fires, there is an increase in oxygenated blood relative to

deoxygenated blood and thus there is more oxygen than required. This causes a change in the

magnetic signal that can be quantified through fMRI (Huettel, Song, & McCarthy, 2004). This

change in magnetic signal is referred to as the blood oxygen level depend (BOLD) signal, which

has been shown to have good spatial and temporal resolution (Matthews & Jezzard, 2004).

Consequently, fMRI enables researchers to produce a neural map of brain activation through

identification of the specific brain regions involved in a given mental process.

The most typical fMRI experimental paradigm utilizes a block design. Within this

paradigm stimuli are presented sequentially through epochs (i.e. "blocks"), typically alternating

between an experimental block and control block. The demands of both conditions are similar,

however, the experimental block is what is utilized to examine the process of interest within the

brain. Following the scan, neural activity during the control block is subtracted from the

experimental block and the remaining brain activity is what is used to isolate the process of

interest. Block designs have been shown to have greater statistical power relative to other fMRI

paradigms, making it a valuable technique to study neural processes of interest (Amaro &

Barker, 2006; Friston et al., 1999). Two fundamental executive processes that may be negatively

influenced by alcohol use during adolescence include response inhibition and cognitive interference, an aspect of sustained attention. Within fMRI block design paradigms, response inhibition can be measured through a Go/No-Go task and cognitive interference can be measured through the Counting Stroop.

Acute alcohol intoxication has been shown to impair performance on both Stroop and Go/No-Go paradigms among healthy social drinkers (Marinkovic, Rickenbacher, Azma, & Artsy, 2012). Among young adults, poor performance on the Go/No-Go task has been shown to predict impulsivity, as well as escalating alcohol use in the future (Fernie et al., 2013). As a result, adolescents with poor capacity for inhibitory responding may be more vulnerable to future alcoholism, which has been suggested to be reflective of a reduced capacity for self-regulation (Saunders et al., 2008). Moreover, individuals who experience difficulty with interference control on the Stroop have been shown to be predictive of both poorer outcomes regarding ability to change (i.e. reduce and/or stop drinking) and subsequent relapse in alcohol dependent individuals (Cox et al., 2007; Marhe et al., 2013). Ultimately, deficits in cognitive control (i.e. response inhibition and interference control) and, by extension, reduced capacity for self-monitoring and self-regulation have been associated with difficulty maintaining abstinence among heavy alcohol users (Saunders et al., 2008; Wilcox et al., 2014).

The Go/No-Go task

The Go/No-Go is a well-known and validated task that taps into response inhibition. The task consists of two conditions of stimuli presented one at a time on a screen: the control condition, "Press for X", and the experimental condition, "Press for every letter except X". Thus, participants learn to respond for a specific letter during the control condition and then are required to refrain from responding to that same letter during the experimental condition. In

healthy control populations, the neural processing required for completion of the Go/No-Go has been shown to be reliant on activation of the anterior cingulate, dorsolateral prefrontal cortex, inferior frontal cortex, inferior parietal lobule, premotor cortex, thalamus, and the caudate using fMRI (Longo et al., 2013; Stevens et al., 2007).

To date, fMRI investigations of the impact of alcohol on response inhibition using the Go/No-Go task have garnered mixed results. In a longitudinal study, Norman et al. (2011) used the Go/No-Go task to examine whether abnormalities in response inhibition would predict regular heavy alcohol use in a sample of adolescents. Participants were imaged at baseline only and no performance differences were found. However, those who transitioned into heavy alcohol users showed a significant reduction in brain activity in a number of areas, which included less activity in the right inferior frontal gyrus, left dorsal and medial frontal areas, bilateral motor cortex, cingulate gyrus, left putamen, bilateral middle temporal gyri, and bilateral inferior parietal lobules. The results of this study highlight the potential of pre-existing vulnerabilities in the developing brain, such as being able to effectively use restraint on risk-taking behaviours, including alcohol use.

In another study, Ahmadi et al. (2013) used the Go/No-Go task to compare differences between light and heavy chronic alcohol users in a sample of young adults (ages 18 to 20). Compared to heavy drinkers, light drinkers (e.g. average of 2.85 drinks per day, 1.08 days per week) showed increased brain activity in a number of regions, including the left superior motor area, bilateral parietal lobe, right hippocampus, bilateral middle frontal gyrus, left superior temporal gyrus, and cingulate gyrus. Performance of heavy drinkers was impaired, showing significantly slower reaction times during the control and experimental trials. Together, these findings suggest that regular heavy alcohol has a widespread depressant effect on brain

functioning, resulting in dysfunction in brain regions that underlie attention and response inhibition. This, in turn, may lead to difficulties in resisting proponent responses and impulsive decision-making in real life situations.

Conversely, a recent study by Ames et al. (2014) found different results when comparing young adult (ages 18 to 22) regular light and heavy alcohol users on a version of the Go/No-Go task using alcohol related cues. In particular, heavy users showed performance impairments, including significantly faster rates of responding and number of errors relative to light users, reflecting difficulties with impulsivity. Unlike the findings of Ahmadi et al. (2013), heavy users also showed significantly more brain activity in the right dorsolateral prefrontal cortex, medial frontal cortex, cingulate, and insula. Interestingly, despite the disparity of results between these studies of light and heavy users, both studies show compensatory brain activation. This is evident through the increased neural activity in areas associated with task performance, as well as the recruitment of additional regions that are not typically associated with the Go/No-Go task, including the temporal gyrus, hippocampus, and insula.

Most recently, Czapla et al. (2017) compared abstinent alcohol-dependent adults to healthy controls on a Go/No-Go task with alcohol-associated stimuli using fMRI following termination from an alcohol detoxification program. There were no significant differences observed between groups in terms of performance measures on the Go/No-Go task though the abstinent alcohol-dependent group showed significantly different brain activity during response inhibition relative to healthy controls. In particular, there was significantly more brain activation in the medial frontal gyrus, medial orbitofrontal cortex, anterior cingulate gyrus, and the occipital areas. The authors postulated that the increase in neural activity in these regions, areas that are important for cognitive control, might be attributable to compensatory strategies to mitigate

possible deficits in attentional and inhibitory processes.

Compensatory recruitment refers to instances where structural damage occurs to a given brain region and alternative routes of functioning are established through recruitment or activation of parallel circuits to meet cognitive demands. Thus, this altered route of neural processing allows the brain to 'compensate' for the damage that has occurred and continue to execute a given cognitive process (Cabeza & Dennis, 2013; Chanraud & Sullivan, 2014; Drummond et al., 2005). Evidence of compensatory recruitment of neural resources as a result of alcohol related brain damage is well documented in neuroimaging studies (Chanraud & Sullivan, 2014; Feldstein-Ewing, Sakhardande, & Blakemore, 2014; Silveri et al. 2011; Sullivan, Harris, and Pfefferbaum, 2010).

In particular, chronic alcohol users show alterations in their neural processes compared to healthy controls when attempting to complete a task that are likely attributable to alcohol related degradation of preferred neural systems. This inefficiency in cortical processing results in increased brain activation in the compromised areas, as well as recruitment of additional adjacent brain regions not typically associated with the type of processing being measured (Chang et al., 2008; Drummond et al., 2005; Sullivan, Harris, and Pfefferbaum, 2010). While this may be helpful in the interim, as cognitive demands increase the change in brain function and increased energy demands that occur through compensatory mechanisms may lead to impeded functioning by interfering with simultaneous processes (Chanraud & Sullivan, 2014). Ultimately, this taxation on the brain may cause impairments in higher-order cognitive processes that impede goal-directed behaviours.

The Counting Stroop task

Bush et al. (1998) developed the Counting Stroop task to use within the fMRI environment, as a measure of cognitive interference. Cognitive interference occurs when simultaneous processing features of a second, intrusive stimulus obstruct the ability to process features of the primary stimulus (Bush et al., 2006). During the task, participants are presented with sets of one to four identical words, such as neutral words in the control condition (e.g. cat, dog, bird, or mouse) or number words in the interference condition (e.g. one, two, three, four). This is followed by indicating the number of words in each set using a four-button response pad, regardless of what the words are. Thus, during the interference condition the written numbers are meant to interfere with a participant's ability to respond, or "count", the amount of stimuli presented in each set. In healthy controls, the task has been shown to be dependent on the brain regions responsible for attentional circuitry, as well as response selection and motor output. This includes the anterior cingulate, dorsolateral PFC (DLPFC), inferior temporal gyrus, superior parietal lobule, and left premotor cortex across a number of studies (Bush et al., 1996; Bush et al., 2006).

While the Counting Stroop itself has not been conducted in studies examining the effect of adolescent alcohol use, findings from research that has utilized other versions of the Stroop task that tap into similar aspects of cognitive interference can be extrapolated for the present purposes. Silveri et al. (2011) compared adolescents who were low-risk and high-risk to develop alcohol abuse, based on a familial history of alcohol dependence, on the color-word Stroop interference task using fMRI. An absence of performance differences was observed between groups, however, high-risk adolescents showed significantly more regional brain activation in both anticipated and additional brain regions, including the right anterior cingulate cortex, right

middle frontal gyrus, left superior frontal gyrus, and right precentral gyrus. Importantly, these results highlight that there are pre-existing vulnerabilities in youth at high-risk for substance abuse, which, in turn, may translate to the brain attempting to sustain performance as cognitive demands rise by increasing activity of compromised and adjacent regions.

Using fMRI, Banich et al. (2007) compared adolescents (ages 14 to 18) with severe substance and conduct problems to healthy controls on the color-word Stroop task. Relative to controls, individuals with severe substance and conduct problems performed similarly on performance measures of the Stroop but showed significantly different brain activity. More specifically, this included increased activation in the superior frontal gyrus, middle and superior temporal gyri, caudate, thalamus, and bilateral activation of the parahippocampal gyrus. Although it remains unclear whether these findings are primarily attributable to the severe substance abuse, conduct problems, or both, these results show a significant attentional dysregulation in the brain of the experimental participants. The significant activation of task-irrelevant regions likely indicates deficits within the prefrontal regions in modulating attentional processes.

Tapert et al. (2004) compared differences between young women (average age 21.44) who were alcohol-dependent to light social drinkers using fMRI with a Stroop-like task containing alcohol-related words. Relative to neutral words, during presentation of alcohol-related words alcohol-dependent women showed increased activation in the left anterior cingulate, left DLPFC, the left inferior frontal gyrus, and bilateral uncus, insula and precuneus. However, there were no performance differences between groups. Together, these results suggest that alcohol-dependent women have increased neural reactivity to alcohol related cues shown by increased activation of brain regions related to attention and reward, which may contribute to the

maintenance of substance abuse problems.

Although the findings summarized from Banich et al. (2007) and Tapert et al. (2004) are pertinent, the results of both studies are limited by the sample population used. In Banich et al. (2007), the results are based on male adolescents admitted to treatment programs for substance use, which also includes other drugs other than alcohol, in addition to significant conduct problems. Comparably, the sample used in Tapert et al. (2004) was based on young adult female participants that met criteria for alcohol dependence. As a result, this highlights a significant gap in the literature on our current understanding of how alcohol use, whether low, moderate, or heavy use, impacts attentional aspects of executive functions in the developing brain.

Purpose of dissertation

Currently, there is little research being conducted on what risk is posed to youth by consuming low to moderate amounts of alcohol, despite the vulnerability of the developing brain and the fact that alcohol related structural damage occurs along a dosage continuum (Paul et al., 2008). The purpose of this research is to address the consequences of low-level alcohol use on two important aspects of executive functioning, response inhibition and cognitive interference, and its associated neurophysiology in young adults. Through examining the effect of regular consumption of small amounts of alcohol beginning in adolescence, we hope to establish what potential risks to youth early drinking may have on the developing brain and functioning later in life. Ultimately, we hope that this preliminary research could be of potential use to Canadian health care initiatives and policies aimed at substance use, as well as educational resources and risk prevention programs to inform youth and their families.

The data in the present thesis are based on young adult participants recruited from the Ottawa Prenatal Prospective Study (OPPS) – a longitudinal study that has followed a cohort of individuals from birth until young adulthood over the past 20 years. This distinct sample of research participants has been providing information on important lifestyle variables, such as education, intelligence quotient (IQ), and socioeconomic status (SES), as well as important developmental variables, like prenatal drug exposure and life course drug use. As such, the OPPS is unlike many other research samples used in drug research, as it permits for the control of many potentially confounding variables, in terms of both lifestyle and prenatal/adolescent drug exposures.

Due to the neuromaturation that occurs in the late teen years and into early adulthood, the OPPS participants were imaged in young adulthood between the ages of 19-21 years. Through the use of this unique sample, the current dissertation aims to examine the distinctive contribution of low-level alcohol use on the neurophysiology of the PFC and associated areas involved in executive functioning. Original data collection included 34 participants who were imaged completing the Counting Stroop and the Go/No-Go tasks. All participants also provided a urine sample and completed a self-report drug use questionnaire on the day of testing, as well as having completed a comprehensive neuropsychological assessment within the past year.

A total of six participants were excluded from the findings presented in this dissertation: one participant was excluded because of structural abnormalities detected from MRI; one participant was excluded due to history of an Axis I disorder; and four participants were excluded after testing positive for cocaine, opiates, and/or amphetamine over the past month through urinalysis and/or self-report drug history questionnaire. More details on the selection criteria are available in subsequent sections.

The resulting sample of 28 participants consisted of 17 low-level alcohol users (9 male, 8 female; mean age = 20; range 19-21 years old) and 11 controls users (5 male, 6 female; mean age = 20; range 19-21 years old). Low-level alcohol use is defined as consumption of an average of 1 to 7 drinks per week (Paul et al., 2008). In the current sample, participants in the alcohol users group had an average of 4.72 drinks/week (SE = 0.74), 1 to 2 times/week, and the average age of their first exposure to alcohol was 14.3 years-old (ages ranging from 12 to 16). Participants of the controls group reported sporadic to no alcohol use. More specifically, 3 controls reported never drinking and the additional 8 controls reported consuming <4 drinks/month on average and had an average age of 15.5 for first alcohol exposure (ages ranging from 13 to 19).

Manuscripts and hypotheses

The present dissertation is comprised of two original manuscripts based on the results of performance on the Counting Stroop and the Go/No-Go in the same sample of OPPS participants across both tasks. In the first original manuscript, young adult low-level alcohol users were compared to controls on the Counting Stroop using fMRI to assess potential differences in cognitive interference between groups. It was hypothesized that there would be no performance differences observed between groups. However, among alcohol-users significant differences in BOLD activity were anticipated for both regions normally associated with the task, as well as adjacent task-irrelevant brain regions reflective of compensatory engagement (Banich et al., 2007; Silervi et al., 2011; Taper et al., 2004).

In the second original manuscript, response inhibition was examined using the Go/No-Go task with fMRI in the same sample of young adult low-level alcohol users and controls. Similarly, it was hypothesized that there would be no performance differences between groups with significant differences expected across groups in regional brain activity. In particular, it was

anticipated that the activation of areas of the brain typically associated with this task of response

inhibition would be increased among alcohol users and that they would also show recruitment of

additional brain regions in order to complete the task (Ahmadi et al., 2013; Ames et al., 2014;

Czapla et al., 2017).

Chapter 2

Effects of low-level alcohol use on cognitive interference:

An fMRI study in young adults

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Abstract

Alcohol consumption is widely known to adversely impact human health. Its neuropathology is largely evident in the cerebellum and frontal lobes, particularly in the immature brains of adolescents and young adults. It may also have a long-lasting impact on executive functioning. The Ottawa Prenatal Prospective Study (OPPS) has followed participants over 20 years, from birth to young adulthood, and has collected data on potentially confounding lifestyle variables, such as prenatal drug exposure and current drug use. The present study investigated the neural activity of 28 young adults from the OPPS using fMRI. The main objective was to discover the impact of regular low-level alcohol consumption on the cognitive interference of these participants, as they performed a Counting Stroop task. Results indicated that, despite a lack of performance differences, young adults who use alcohol on a regular basis differ significantly from controls with respect to their neural activity as they perform this task. Areas that were significantly more activated in users compared to controls included the: cerebellum, thalamus, fusiform gyrus, prefrontal cortex, and precuneus. The observed activity suggests a significant impact of early alcohol use on neurophysiology despite relatively low levels of alcohol consumption.

Introduction

Despite the fact that alcohol consumption is widely known to adversely impact human health, research is only beginning to unveil its ability to disrupt developmental and functional processes in the brain (World Health Organization, 2007). Indeed, individuals diagnosed with alcoholism show significant decreases in gray and white matter volumes and increases in sulcal and ventricular volumes (Pfefferbaum et al., 1992). Both effects worsen for heavy drinkers with age when drinking continues and there is also significant and continued axonal and neuronal injury over time (Pfefferbaum et al., 1995, 1997; Harper, 2009). This neuropathology of alcoholism is particularly evident in the frontal lobes, which are especially vulnerable to damage in immature brains of adolescents and young adults (Faden and Goldman, 2005; Meyerhoff et al., 2005). These structural and cellular changes are in line with the evidence accumulated demonstrating that prolonged use of alcohol has a significant negative impact on adult brain function, predominantly with respect to executive functioning (for a review, see Moselhy et al., 2001).

It is also now well documented that the deleterious impact of alcohol differs among adolescents and adults, with the developing brain of adolescents being more vulnerable to the harmful effects of alcohol (Guerri and Pascual, 2010; Squeglia et al., 2012). However, there appears to be a marked skew in the focus of this initial research, which has predominantly examined the effects of chronic heavy alcohol use and high level drinking (Pfefferbaum et al., 2001; Tapert et al., 2001, 2004; De Bellis et al., 2005; Schweinsburg et al., 2005, 2010; Sequeglia et al., 2012; Wetherill et al., 2013). Little attention has been paid to the functional consequences of light to moderate drinking behaviours despite evidence that structural damage is accumulated along a dosage continuum (Paul et al., 2008). Further research on the effects of

alcohol on the developing brain as well as a consensus on how to categorize the levels and patterns of alcohol consumption among adolescents would assist in understanding these functional consequences in the future.

Since its inception, the Stroop task (Stroop, 1935) has proven to be a reliable and valid measure of executive function, and is considered to be a criterion measure of cognitive interference (Lezak, Howieson, & Loring, 2004; Mead et al., 2002). As a measure of executive functioning, it has been used to discriminate patients with frontal lobe dysfunction from controls (Perret, 1974; Demakis, 2004), and has also been used to demonstrate the extent of neural maturation across developmental stages (Adleman et al., 2002; Marsh et al., 2006). Functional magnetic resonance imaging (fMRI) has been used to identify regions of the brain involved in the Stroop effect. These regions include the cingulate gyrus, prefrontal cortex and parietal regions, though activity in these areas differs slightly with different task variations (Peterson et al., 2002; Banich et al., 2000a, 2000b; Milham et al., 2003).

Silveri et al. (2011) used fMRI to examine performance on a Stroop Interference task in adolescents at high-risk for alcohol abuse based on a familial history of alcohol dependence. Compared to low-risk individuals, high-risk individuals showed greater recruitment of a network of frontal lobe regions during performance on this task of inhibition. This greater recruitment of frontal brain regions in high-risk individuals is consistent with previous research suggesting the brain will attempt to maintain performance on cognitively demanding tasks by increasing activation in the compromised and adjacent brain regions (Chang et al., 2008; Drummond et al., 2005). Moreover, these results reflect the view in the current literature that neural vulnerabilities exist in at risk individuals even prior to the initiation of substance use (Everitt et al., 2008; Wetherill et al., 2013).

Banich and colleagues (2007) used fMRI to compare adolescents with severe substance and conduct problems to controls on the color-word Stroop task. Results indicated clear group differences with respect to brain activity, with patients demonstrating increased activity, compared to controls, in the left hemisphere, including the superior frontal gyrus, middle and superior temporal gyri, caudate and thalamus, and bilaterally in the parahippocampal gyrus. Another fMRI study by Tapert et al., (2004), examined differences in brain activity in alcohol-dependent young women versus light social drinkers in response to alcohol stimuli in a Stroop-like task. Compared to controls, alcohol dependent women had increased neural activity in the left anterior cingulate, the left dorsolateral prefrontal cortex (DLPFC), the left inferior frontal gyrus, and bilateral uncus, insula and precuneus. Although pertinent, results from both of these fMRI studies are confounded by the sample population. For example, the Banich et al (2007) patients were all male and were in treatment programs for both substance abuse (of potentially more than just alcohol) and conduct disorder. Similarly, Tapert et al., (2004) used only females with heavy substance abuse.

A variant of the original Stroop task, the Counting Stroop, has been developed for use in fMRI studies to minimize unfavourable head movements in the scanner that often occur with verbal responses (Bush et al., 1998). The primary difference in the task is that participants respond using button response pads, and report the number of words in a group of stimulus words. Using healthy controls, neural activity during the Counting Stroop was shown to be consistent with prior research on the original Stroop, demonstrating activity in the: anterior cingulate, middle frontal gyrus, left precentral gyrus, left premotor cortex, inferior temporal gyrus, and superior parietal lobule.

Given that alcohol use is frequently accompanied by drug use (legal or illicit), especially in adolescents and young adults (WHO, 2007), it is of particular importance to control for such confounds when assessing performance and brain activity during a task like the Counting Stroop. In light of this, the present paper examines data from the Ottawa Prenatal Prospective Study (OPPS), a longitudinal project that has followed participants over 20 years, from birth, and has collected data on potentially confounding lifestyle and drug exposure variables. This unique cohort permits the examination of the neurobehavioural effects on executive functioning resulting from alcohol use, while controlling for adolescent/young adult drug use and prenatal drug exposure confounds, as well as other lifestyle variables, such as socioeconomic status.

At present, there has been little fMRI research on the resulting impact of alcohol on cognitive development and functioning in young adults, and even fewer studies have controlled for other drugs of use and abuse. In the present study, fMRI was used to examine the effects of low-level alcohol use on executive functioning, particularly cognitive interference, in a sample of OPPS participants. It was anticipated that there would be no performance differences between young adult alcohol users and controls while completing the Counting Stroop. Given the areas activated during the Counting Stroop in healthy controls and the development of the PFC at the critical time of neurodevelopment when regular alcohol consumption started, it was hypothesized that areas of the brain related to executive functioning would be negatively impacted by regular alcohol consumption while performing the Counting Stroop. In particular, we anticipated increased neural activity in young adult alcohol users compared with controls within the anterior cingulate, parietal and prefrontal cortices, although these results would likely be less extreme than those observed in chronic heavy drinkers.

Methods

Participants

Fifty participants from the OPPS were randomly contacted until a sample of thirty-four available and suitable youth were recruited. These thirty-four participants provided informed consent and were imaged, regardless of sex or drug exposures. Inclusion criteria required that participants were at least 19 years of age (i.e. the legal drinking age in Ontario), were right handed, had English as their first language, and had completed a recent comprehensive neuropsychological test battery. All participants met fMRI compatibility criteria, including no metal implants, no pacemaker, no recent surgery, suitable vision for viewing stimuli, and no previous metal in eyes. Participants were also excluded if they had previously experienced a head injury with loss of consciousness. Participants were excluded if they tested positive for cocaine, opiates or amphetamines in their urine, or self-reported regular use of any of these drugs (defined as once/month or more; 4 participants). Participants were also excluded if they had a history of an Axis I diagnosis based on the DSM-IV-TR (one participant), any structural abnormalities were detected in their MRI scan (one participant). No participants included in the current study met diagnostic criteria for conduct disorder.

The purpose of the present investigation was to examine the influence of regular consumption of low amounts of alcohol on the neurophysiology of cognitive interference relative to individuals who drink irregularly, if at all. Therefore, participants were assigned to the alcohol users group if they met the cut-off criteria of consuming an average of >1 drink/week over the past year. The remaining participants who reported consuming an average of <1 drink/week and <5 drinks/month were assigned to the control group. These criteria resulted in data from 17 alcohol users (9 male, 8 female, mean age of 20.06 [SE=0.23], range 19-21 years) and 11

controls (5 male, 6 female, mean age of 20.09 [SE=0.25], range 19-21 years) being included for

analyses. Alcohol use was reported as the number of alcoholic drinks consumed per week. Over

the previous year, users reported consuming an average of 4.72 drinks/week (SE = .74) over 1 to

2 times/week, with first alcohol exposure ranging from age 12-16 for this group (mean age of

14.3). The controls reported only sporadic use, if any (3 did not drink alcohol at all while the

other 9 averaged less than 4 drinks/month), with a first exposure to alcohol ranging from 13-19

years old (mean age of 15.5). In terms of nicotine use, 7 participants within the alcohol users

group reported using nicotine within their lifetime, with 6 of them reporting current nicotine use.

Within the control group, 4 participants reported lifetime nicotine use, with 3 of these

participants being current nicotine users.

Clinical and drug use assessments

Psychological and neuropsychological measures had been administered to participants at

most one year prior to testing, which included the Wechsler Adult Intelligence Scale-III (WAIS-

III) (Wechsler, 1997) and the Computerized Diagnostic Interview Schedule for Children (Bacon,

1997), which assessed for current psychiatric illness based on DSM-IV criteria. Parents also

completed the Conners' Parent Rating Scale (Goyette et al., 1978) and provided information on

socioeconomic status. No significant differences were observed between current users and

controls on these scales, and therefore they were not included in the analyses (Table 1).

Moreover, no significant differences were observed between current alcohol users and controls

for socioeconomic status (parental education and family income), thus it was not included in the

analyses (Table 1).

As a means to control for current drug use, participants provided urine samples upon

arrival at the MRI scanner. Samples were screened for cannabis, amphetamines, opiates, cocaine,

nicotine (via cotinine), crack, heroin, lysergic acid diethylamide, solvents, steroids, and

mushrooms. The samples were also tested for creatinine, which was used as an indicator of urine

dilution. All participants also completed a self-report drug questionnaire, requesting information

on alcohol, marijuana, nicotine, mushrooms, amphetamines, crack, cocaine, tranquilizers, heroin,

lysergic acid diethylamide, solvents, and steroids.

Imaging protocol

Data acquisition. Each participant was involved in one imaging session on a 1.5 Tesla

Siemens Magnetom Symphony MR scanner. Participants lay supine with their head secured in a

custom head holder while a conventional T1-weighted spin echo localizer was acquired and used

to prescribe a subsequent 3D FLASH (TR/TE 11.2/21 ms, flip angle 60°, field of view (FOV)

$26{\times}26$ cm^2, $256{\times}256$ matrix, slice thickness 1.5 mm) volume acquisition. Whole brain blood

oxygen level dependent (BOLD) fMRI was performed using a T2*-weighted echo planar pulse

sequence (TR/TE 3000/40 ms, flip angle 90°, FOV $24{\times}24$ cm^2, $64{\times}64$ matrix, slice thickness 5

mm, 27 axial slices, bandwidth 62.5 kHz).

Equipment. Stimuli were presented on a screen located at the entrance of the magnet,

which participants viewed through a mirror mounted on the head coil. To minimize head

movements in the scanner, participants responded using a 4-button MRI-compatible fibre optic-

response pad (Lightwave Medical, Vancouver, British Columbia, Canada) using their right hand.

All lighting in the scanning room was off. Visual and Auditory Presentation Package (VAPP)

was used to present stimuli and record responses.

Procedure and design. The Counting Stroop task used in this study was the same as that

used in the original article by Bush and colleagues (1998). Stimuli were number or animal words

printed in white on a black background. Words were common words from each of the two semantic categories, and were balanced for word length. Participants were presented with 1-4 identical words, horizontally presented one above another, and asked to report, using the appropriate button on the response pad, the number of words observed for each group (index finger for one word, middle finger for two words etc.). This task was a block design with two conditions. In the congruent trials stimulus words were the names of common animals (i.e., dog, cat, bird or mouse). For incongruent trials, stimulus words were number words (i.e. "one", "two", "three", or "four"), each trial including a different number of words than the word itself.

The task consisted of eight 30 s blocks of the congruent words, which were alternated with eight incongruent 30 s blocks for a total task time of 8 minutes. Participants completed 20 trials during each block, which yielded 160 total trials. The interstimulus interval was 1.5 s. Block order was counterbalanced across scans. Please see Appendix A, Figure 1 for a diagram of the task paradigm. Participants were instructed to respond as quickly and accurately as possible and to do their best. Participants were required to view the task outside the scanner and perform one block of each condition prior to the imaging to guarantee that each participant was able to perform the task accurately.

Cognitive performance parameters and analyses. Reaction time for each response and errors of commission were recorded. Errors of commission included any inaccurate responses. Mean reaction times were calculated for both the 'Animal' and the 'Number' conditions for all accurate responses occurring within 900 ms of stimulus presentation. Separate analyses of variance were performed on reaction time data, errors of commission, and errors of omission using current nicotine, current marijuana, and prenatal alcohol as covariates (Dahlgren, Sagar, Racine, Dreman, & Gruber, 2016; Froelinger, Modlin, Wang, Kozink, & McClernon, 2012;

Mattson, Crocker, & Nguyen, 2011; Sager et al., 2016; Wignall, & de Wit, 2011). All statistical

analyses were performed in Statistical Package for Social Sciences (SPSS).

Image post-processing. The functional brain images were realigned to correct for motion

by employing the procedure of Friston and colleagues (1995), using Statistical Parametric

Mapping (SPM8) software. The motion correction did not exceed 1 mm for any participant.

Images were spatially normalized to match the echo planar imaging (EPI) template provided in

SPM8. Images were then smoothed with an 8 mm full-width at half-maximum Gaussian filter.

The observed time course of image intensity in each voxel was temporally filtered to remove

frequency components below 0.36 Hz and fitted (using the general linear model) to a model

hemodynamic response consisting of sequential contributions from sequential epochs. The

hemodynamic response to each type of epoch was convolved with a 6 s filtered boxcar waveform

and was subjected to the same high-pass temporal filter as the observed time course.

Whole brain analyses with SPM8. Fixed effects analyses were performed on all

participants individually and then statistical parametric maps were obtained for the two groups:

current alcohol users and controls. Contrast images were calculated for these analyses for the

contrast of interest: 'Numbers' minus 'Animals'. Subsequently, these images were used for

second-level random effects analyses, allowing for a comparison between groups.

Multiple independent samples t-tests were conducted at a set threshold of $p_{uncoorr} = 0.001$,

with a cluster-wise correction at $p_{FWE} = 0.05$ to assess group differences in neural activity during

the Counting Stroop, between current alcohol users and controls, were performed, manipulating

the covariates that were included in the analysis. The final reported results are those from the

comparison of current alcohol users and controls using prenatal alcohol, current marijuana and

current nicotine use as covariates. These covariates were not significantly correlated with current

alcohol use so were used as covariates to ensure that no other current or prenatal drug effects were responsible for the observed results. Additional analyses were performed without those participants who smoked marijuana on the day of testing (n=3, no participant smoked more than 1 joint or within 6 hours of the scanning). No differences in results were observed with or without these participants, suggesting that the obtained results were not due to acute effects of marijuana.

Results

Participant characteristics

The two groups were significantly different for weekly current alcohol use ($F = 35.49$, $p<0.000$), however, there were no significant differences between groups based on other drug exposures. Table 2 summarizes the prenatal and current drug exposure of participants for both current alcohol users and controls. No participant, from either group, reported consuming alcohol on the day of testing, eliminating the possibility of the results representing acute alcohol effects rather than regular use effects on neural processing. There were 2 participants from the non-using alcohol group that were regular marijuana smokers, defined as at least one joint/day, while there were 4 alcohol users that met this criterion. Similarly, nicotine use was considered for each group with 5 of the alcohol users and 4 of the controls smoking cigarettes on a regular basis (everyday). No other illicit drug use was reported or observed in urine for any participant included in the results. All analyses were performed with and without these participants to eliminate marijuana and nicotine as the reason for the results obtained. No differences were observed between these analyses and thus only the results including all participants are reported below.

Drug questionnaire and urine sample data

All metabolite concentrations were adjusted for creatinine to control for urine dilution. The quantity of drug used over the week prior to testing and over the past month was compared with urine sample results for validity of current use. The Pearson correlation between the urine samples for cannabis levels and drug questionnaire was 0.97 ($p<0.001$), while that for nicotine (cotinine/creatinine) was 0.91 ($p<0.001$). This high correspondence validated the use of the drug questionnaire for current drug use and history.

Performance data

After controlling for prenatal alcohol, current marijuana, and current nicotine exposure, there were no significant performance differences between current alcohol users and controls on reaction time, errors of commission, or the Stroop effect (i.e. Incongruent - Congruent trials) based on ANOVA results (see Table 3).

fMRI data

Investigating the within group, first level fixed effects analyses, revealed an expected pattern of activity for both groups, including increased activation for the incongruent minus congruent contrast in the DLPFC, superior temporal gyrus, superior parietal lobule, anterior cingulate and premotor and primary motor cortices. Although both groups showed a similar pattern of activity in anticipated areas for the Stroop effect, there were significant differences between groups when performing the random effects group comparison.

The most robust results are presented at threshold $p_{crit}<0.001$ uncorrected with $p_{FWE} = 0.05$ cluster-wise correction. These results were the significantly increased neural activity observed in alcohol users compared to controls in the left precuneus (x, y, z = -24 -51 20, z =

3.88, $p = 0.025$), the left thalamus (x, y, z = -24 -27 5, z = 3.26, $p = 0.025$), the left fusiform

gyrus (x, y, z = -21 -75 -5, z = 3.21, $p = 0.025$) and the left anterior cerebellum (x,y,z = -12 -45 -

25, z = 2.85, $p = 0.025$) (Figure 1A-C). These regions made up a cluster of 958 activated voxels.

Alcohol users also showed significantly more activation than controls in another large cluster of

1067 voxels including the right precuneus (x,y,z = 9 -51 70, z = 3.23, $p = 0.014$), and right

superior parietal lobule (x, y, z = 15 -57 60, z = 3.0, $p = 0.014$) (Figure 1D-E). All results were

significant after correcting for multiple comparisons at $p = 0.05$ and controlling for prenatal

alcohol exposure, current marijuana, and current nicotine use. These drug exposures were used

as covariates to ensure the observed results were as representative of alcohol alone, though we

acknowledge that completely removing the co-occurring impact of these variables is not entirely

possible.

In addition, although uncorrected for multiple comparisons at the cluster level (p_{uncorr} =

0.05), alcohol users showed more activity than controls during the Number – Animal contrast in

the left PFC (x, y, z = -36 30 5, z = 3.5, $p = 0.05$) and the right precentral gyrus (x, y, z = 45 6

45, z = 3.13, $p = 0.031$; Figure 1F), right superior temporal gyrus (x,y,z = 57 -60 15, z = 3.57, p

= 0.029, in a cluster of 73 voxels), and the right middle frontal gyrus (x, y, z = 48 9 40, z = 3.31,

p uncorrected = 0.03, in a cluster of 72 voxels). There was also significantly more activity in

alcohol users than controls in the cingulate gyrus (x,y,z = -24 18 40, z = 3.08, $p = 0.023$, cluster

of 294 voxels).

Discussion

The present study examined the fMRI BOLD response to a Counting Stroop

attention/interference task contrasting young adults who consumed alcohol on a regular basis, in

the form of low-risk drinking, and those who were not regular users. Despite similar task

performance, there were brain regions that were significantly more activated in the alcohol users than controls when challenged by the cognitive conflict processing of the task.

Interestingly, despite regularly consuming only low-level amounts of alcohol, the alcohol users group still revealed more extensive activation patterns than controls in the cerebellum, thalamus, fusiform gyrus, superior parietal lobule, precuneus, as well as significant differences in the prefrontal cortex, cingulate gyrus and superior temporal gyrus. These results are unique given the amount of information available for these participants from the OPPS, and the ability to control for relevant variables, such as previous drug exposure.

The most substantial difference between groups was observed in the left fusiform gyrus, a region not typically associated with performance of the Counting Stroop in healthy controls, though some studies have found increased activity in this region in other variants of the Stroop effect (Bush et al., 2006; Bush et al., 1998; Kronhaus et al., 2006; Roth et al., 2006). The fusiform gyrus is typically considered part of the extrastriate cortex and is associated with visual object processing, as well as visual attention. It is possible that the alcohol group in the present study required more visual focus to perform the task accurately. This is also perhaps why there was increased activity in the precuneus and more lateral superior parietal lobule activity in alcohol users compared to controls. With respect to attention, the posterior/parietal system is thought to be involved in attending to attributes of task-relevant stimuli (Banich et al., 2000b), and research has shown this area to be specifically involved in uncued shifts of spatial and non-spatial attention (Makino et al., 2004). Thus, it may be that alcohol users require greater attention, and thus greater neural activity, than controls to perform this task appropriately. There is a spatial component to the Counting Stroop as one word on the screen takes up much less space than if 4 words are on the screen. This strategic method of performing the task may have

been eliminated by the subtraction of congruent blocks from incongruent blocks but perhaps this processing is affected by alcohol consumption.

These results correspond with results from Tapert et al., (2004) who observed increased bilateral parietal and precuneus activity in adolescents with alcohol use disorders during a spatial working memory task. Schweinsburg et al., (2010) also observed this type of enhanced activity in parietal areas during a verbal learning task in heavy episodic drinking adolescents. Both studies observed decreases in activity in the alcohol using groups of adolescents studied in certain areas where the present paper observed increases in activity only (including the cerebellum) (Tapert et al., 2004; Schweinsburg et al., 2010). Some possible reasons for these differences in results are that the participants from the other studies were younger than those in the present study, were heavy consumers of alcohol, and were performing different cognitive tasks.

Our findings are supported by Wetherill et al. (2013), who conducted a longitudinal study that followed adolescents before and after initiation of heavy drinking. Prior to alcohol use, participants showed less recruitment of frontal, parietal, subcortical, and cerebellar regions compared to controls and at follow-up there was significantly greater activation of these areas while performing a task of response inhibition. This implies that the brains of heavy alcohol consumers were attempting to compensate for the cognitive demands of the task during performance, which was not observed in controls. These findings parallel the patterns of brain activation observed among low-level alcohol consumers in the current study, such as the cerebellum, middle frontal gyrus, and parietal cortices, despite differences in task type and level of alcohol use. This may also suggest that regular alcohol consumption, whether in low or high

quantities, is particularly damaging to these areas of the brain and possibly along a dosage continuum.

Compared to the other fMRI studies that have examined the Stroop task in adolescent populations with substance abuse, the results from the present paper are relatively consistent. Tapert and colleagues (2004) examined a task of attentional interference using alcohol-related stimuli in alcohol dependent young women. The control group was composed of light social drinkers, drinking an average of approximately 7 drinks per month. It was not surprising that results from our alcohol users more closely resembled this control group, who also showed increased activity in the right PFC. Conversely, alcohol-dependent women demonstrated increased activity in areas not observed in our sample of drinkers. Given that our sample had not met criteria for a substance abuse disorder, this is not surprising. Another study, by Banich and colleagues (2007), examined the Stroop task in a group of adolescents with severe substance abuse and conduct disorder and reported similar, more extensive parallels with our findings. In particular, in both studies performance equivalence was observed between users and controls, but users activated a greater range of brain structures for incongruent versus congruent trials. These regions were consistent with those typically activated in the Stroop task, although in both studies users engaged prefrontal regions outside of the DLPFC, to obtain performance equivalence.

In addition to the fusiform gyrus and parietal lobe differences in activation between groups in the present study, alcohol users also showed increased activation in structures typically affected by chronic alcohol use, particularly the cerebellum (Smith and Fein, 2011), thalamus (Harding et al., 2000), and prefrontal cortex (Abernathy et al., 2010). Paul et al. (2008) examined total cerebral brain volumes in a healthy community sample in low, moderate and high drinkers in addition to abstainers and previous drinkers. The researchers found that alcohol consumption

was associated with decreased brain volumes even in those who consume low amounts defined as 1-7 drinks per week, and despite cardiovascular benefits. Our study suggests an impact of even low amounts of alcohol on neural functioning. Unfortunately, this study did not investigate changes to the structural integrity of white matter or volume measures but this will be added to the research protocol for further imaging studies with the OPPS, as it is clear that compromised frontal-posterior circuitry may well contribute to altered cognitive processes (Bava et al., 2009).

The prefrontal, thalamic, and cerebellar structures comprise a neural pathway that is hypothesized to modulate executive functioning (Herting et al., 2011; Heyder et al., 2004). The cognitive and behavioural deficits observed in alcoholics are believed to result from the disruption of this *frontocerebellar* circuit (Sullivan et al., 2003). Initial deficits linked to alcohol use are hypothesized to target the cerebellum (Kouzoukas et al., 2013; Parks et al., 2002). Therefore, the activity observed in this study, in this neural circuit, may reflect the early stages of neurocognitive dysfunction in young alcohol users. Moreover, since the cerebellum and frontal cortices mature relatively late in the course of neuronal development (Diamond, 2001), young adults may be particularly at risk for developmental and functional changes related to alcohol consumption. Although a variation of the Stroop task was used in this study, the interference is comparable, especially since the process of counting is required in addition to reading in incongruent conditions. Thus, the increased activation in frontal regions (Schweinsburg et al., 2005) and the cerebellum (Diamond, 2001) could also suggest a greater difficulty with general task demands and a resulting need for increased concentration in users compared to controls. It would be interesting to investigate whether these regions could compensate with further increases in task difficulty.

This study provides support for previous findings of the effects of early regular use of alcohol on interference/attentional neural processing. The strength of the paper is the use of the OPPS sample and thus the ability to control for lifestyle variables, including drug use over the lifespan. This unique methodology strengthens the validity of the results and provides outcomes that are able to shed light on select contributions of alcohol on neural processing. The results encourage the importance of further research on the potentially damaging effects of low levels of alcohol consumption in adolescents on future neural development that may lead to possible long-term detrimental effects.

There are several important limitations inherent in this study relating to sample size and the task design itself. In terms of size, the number of participants in each group was relatively small and replication would be essential. Due to the low quality of the structural scans acquired, brain volume could not be assessed, which makes it unclear whether differences observed in patterns of brain activation were related to structural differences between alcohol users and controls. It may have also been helpful to have some rest blocks between incongruent and congruent conditions as the hemodynamic response may not have had enough time to equilibrate in the interim. However, the ability to detect such changes is the benefit of block design fMRI experiments as opposed to event-related ones.

Another limitation of the study is the use of the Counting Stroop itself. There is an additional working memory component of mapping the number of words to the finger representing each number. Additionally, the semantic content of the words (i.e. animals or numbers) differs across the two experimental conditions, and therefore the subtraction will contain not only the effects of cognitive interference (number of words vs. the written numbers)

but also word meaning and semantics. As such, group differences in brain activation may be driven by differences in semantic processing and not attentional processing.

Conclusions

This study provides support for a significant neural impact of small amounts of regular alcohol use in young adulthood. Despite this low-level of alcohol consumption, significant alterations in brain activity were observed during a task requiring self-regulatory control to inhibit responses, even when no performance deficits were present. Further levels of difficulty for the attentional circuitry required to perform the task may not be able to compensate for the altered activation pattern. Further fMRI studies using the unique OPPS sample, that allowed for the control of prenatal and current exposure to drugs, among other lifestyle variables, will be important to further isolate the impact of alcohol use in young adults.

Disclaimer

Please note that the article presented in the present dissertation has been adapted since its original publication in 2015. This was done in order to improve the quality of the manuscript and make it consistent with the methods used in the subsequent Go/No-Go manuscript. In particular, the covariates used in the performance measures of the Counting Stroop were changed to current alcohol, current nicotine, and prenatal alcohol only for consistency with the covariates used in the functional imaging analyses. Moreover, in the original manuscript the control group was previously labeled as non-users.

References

Abernathy, K., Chandler, L. J., & Woodward, J. J. (2010). Alcohol and the prefrontal cortex. *International Review of Neurobiology*, *91*, 289–320. doi:10.1016/S0074-7742(10)91009-X

Adleman, N. E., Menon, V., Blasey, C. M., White, C. D., Warsofsky, I. S., Glover, G. H., & Reiss, A. L. (2002). A developmental fMRI study of the Stroop color-word task. *NeuroImage*, *16*(1), 61–75. doi:10.1006/nimg.2001.1046

Bacon, W. (1997). *NIMH-Computerized Diagnostic Interview Schedule for Children-version IV (C-DISC 4)*. (New York, NY: Columbia University).

Banich, M. T., Crowley, T. J., Thompson, L. L., Jacobson, B. L., Liu, X., Raymond, K. M., & Claus, E. D. (2007). Brain activation during the Stroop task in adolescents with severe substance and conduct problems: A pilot study. *Drug and Alcohol Dependence*, *90*(2-3), 175–82. doi:10.1016/j.drugalcdep.2007.03.009

Banich, M. T., Milham, M. P., Atchley, R. A., Cohen, N. J., Webb, A., Wszalek, T., Brown, C. (2000a). Prefrontal regions play a predominant role in imposing an attentional "set": evidence from fMRI. *Brain Research. Cognitive Brain Research*, *10*(1-2), 1–9.

Banich, M. T., Milham, M. P., Atchley, R., Cohen, N. J., Webb, A., Wszalek, T., Magin, R. (2000b). fMri studies of Stroop tasks reveal unique roles of anterior and posterior brain systems in attentional selection. *Journal of Cognitive Neuroscience*, *12*(6), 988–1000.

Bava, S., Frank, L. R., McQueeny, T., Schweinsburg, B. C., Schweinsburg, A. D., & Tapert, S.

F. (2009). Altered white matter microstructure in adolescent substance users. *Psychiatry*

Research, *173*(3), 228–37. doi:10.1016/j.pscychresns.2009.04.005

Bush, G., Whalen, P. J., Rosen, B. R., Jenike, M. A., McInerney, S. C., & Rauch, S. L. (1998).

The counting Stroop: an interference task specialized for functional neuroimaging--

validation study with functional MRI. *Human Brain Mapping*, *6*(4), 270–82.

Bush, G., Whalen, P. J., Shin, L. M., & Rauch, S. L. (2006). The counting Stroop: a cognitive

interference task. *Nature Protocols*, *1*(1), 230–3. doi:10.1038/nprot.2006.35

Chang, L., Yakupov, R., Nakama, H., Stokes, B., & Ernst, T. (2008). Antiretroviral treatment is

associated with increased attentional load-dependent brain activation in HIV patients.

Journal of Neuroimmune Pharmacology : The Official Journal of the Society on

NeuroImmune Pharmacology, *3*(2), 95–104. doi:10.1007/s11481-007-9092-0

Dahlgren, M., Sagar, K., Racine, M., Dreman, M., & Gruber, S. (2016). Marijuana Use Predicts

Cognitive Performance on Tasks of Executive Function. *Journal of Studies on Alcohol and*

Drugs, 77, 298-308.

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 and Experimental Research, *29*(9), 1590–600.

Demakis, G. J. (2004). Frontal lobe damage and tests of executive processing: a meta-analysis of

the category test, stroop test, and trail-making test. *Journal of Clinical and Experimental*

Neuropsychology, *26*(3), 441–50. doi:10.1080/13803390490510149

Diamond, A. (2001). Close interrelation of motor development and cognitive development and

of the cerebellum and prefrontal cortex. *Child Development*, *71*(1), 44–56.

Drummond, S. P. A., Meloy, M. J., Yanagi, M. A., Orff, H. J., & Brown, G. G. (2005).

Compensatory recruitment after sleep deprivation and the relationship with performance.

Psychiatry Research, *140*(3), 211–23. doi:10.1016/j.pscychresns.2005.06.007

Everitt, B. J., Belin, D., Economidou, D., Pelloux, Y., Dalley, J. W., & Robbins, T. W. (2008).

Review. Neural mechanisms underlying the vulnerability to develop compulsive drug-

seeking habits and addiction. *Philosophical Transactions of the Royal Society of London.*

Series B, Biological Sciences, *363*(1507), 3125–35. doi:10.1098/rstb.2008.0089

Faden, V.B., & Goldman, M. (2005). The effects of alcohol on physiological processes and

biological development. *Alcohol Research and Health*, *28* (3), 125–132.

Friston, K. J., Ashburner, J., Frith, C. D., Poline, J.-B., Heather, J. D., & Frackowiak, R. S. J.

(1995). Spatial registration and normalization of images. *Human Brain Mapping*, *3*(3), 165–

189. doi:10.1002/hbm.460030303

Froeliger, B., Modlin, L., Wang, L., Kozink, R., & McClernon, V. (2012). Nicotine withdrawal

modulates frontal brain function during an affective Stroop

task. *Psychopharmacology, 220*, 707-718.

Goyette, C. H., Conners, C. K., & Ulrich, R. F. (1978). Normative data on revised Conners

    Parent and Teacher Rating Scales. *Journal of Abnormal Child Psychology*, *6*(2), 221–36.

Guerri, C., & Pascual, M. (2010). Mechanisms involved in the neurotoxic, cognitive, and

    neurobehavioral effects of alcohol consumption during adolescence. *Alcohol*, *44*(1), 15–26.

    doi:10.1016/j.alcohol.2009.10.003

Harding, A., Halliday, G., Caine, D., & Kril, J. (2000). Degeneration of anterior thalamic nuclei

    differentiates alcoholics with amnesia. *Brain : A Journal of Neurology*, *123*(Pt 1), 141–54.

Harper, C. (2009). The neuropathology of alcohol-related brain damage. *Alcohol and*

    Alcoholism, *44*(2), 136–40. doi:10.1093/alcalc/agn102

Harper, C. (2007). The neurotoxicity of alcohol. *Human & Experimental Toxicology*, *26*(3), 251–

    7. doi:10.1177/0960327107070499

Herting, M. M., Fair, D., & Nagel, B. J. (2011). Altered fronto-cerebellar connectivity in

    alcohol-naïve youth with a family history of alcoholism. *NeuroImage*, *54*(4), 2582–9.

    doi:10.1016/j.neuroimage.2010.10.030

Heyder, K., Suchan, B., & Daum, I. (2004). Cortico-subcortical contributions to executive

    control. *Acta Psychologica*, *115*(2-3), 271–89. doi:10.1016/j.actpsy.2003.12.010

Kouzoukas, D. E., Li, G., Takapoo, M., Moninger, T., Bhalla, R. C., & Pantazis, N. J. (2013).

    Intracellular calcium plays a critical role in the alcohol-mediated death of cerebellar granule

    neurons. *Journal of Neurochemistry*, *124*(3), 323–35. doi:10.1111/jnc.12076

Kronhaus, D. M., Lawrence, N. S., Williams, A. M., Frangou, S., Brammer, M. J., Williams, S.

    C. R., Phillips, M. L. (2006). Stroop performance in bipolar disorder: further evidence for

    abnormalities in the ventral prefrontal cortex. *Bipolar Disorders*, *8*(1), 28–39.

    doi:10.1111/j.1399-5618.2006.00282.x

Lezak, M.D., Howieson, D.B., & Loring, D.W. (2004). *Neuropsychological assessment (4th ed.)*.

    (New York, NY: Oxford University Press).

Marsh, R., Zhu, H., Schultz, R. T., Quackenbush, G., Royal, J., Skudlarski, P., & Peterson, B. S.

    (2006). A developmental fMRI study of self-regulatory control. *Human Brain Mapping*,

    27(11), 848–63. doi:10.1002/hbm.20225

Mattson, S.N., Crocker, N., & Nguyen, T.T. (2011). Fetal alcohol spectrum disorders:

    neuropsychological and behavioural features. *Neuropsychological Review*, *21*, 81-101.

Mead, L. A., Mayer, A. R., Bobholz, J. A., Woodley, S. J., Cunningham, J. M., Hammeke, T. A.,

    & Rao, S. M. (2002). Neural basis of the Stroop interference task: response competition or

    selective attention? *Journal of the International Neuropsychological Society : JINS*, *8*(6),

    735–42.

Meyerhoff, D. J., Bode, C., Nixon, S. J., de Bruin, E. A., Bode, J. C., & Seitz, H. K. (2005).

    Health risks of chronic moderate and heavy alcohol consumption: how much is too much?

    Alcoholism, Clinical and Experimental Research, *29*(7), 1334–40.

Milham, M. P., Banich, M. T., & Barad, V. (2003). Competition for priority in processing

    increases prefrontal cortex's involvement in top-down control: an event-related fMRI study

    of the stroop task. *Brain Research. Cognitive Brain Research*, *17*(2), 212–22.

Moselhy, H. F., Georgiou, G., & Kahn, A. (2001). Frontal lobe changes in alcoholism: a review

    of the literature. *Alcohol and Alcoholism (Oxford, Oxfordshire)*, *36*(5), 357–68.

Parks, M. H., Dawant, B. M., Riddle, W. R., Hartmann, S. L., Dietrich, M. S., Nickel, M. K., …

    Martin, P. R. (2002). Longitudinal brain metabolic characterization of chronic alcoholics

    with proton magnetic resonance spectroscopy. *Alcoholism, Clinical and Experimental

    Research*, *26*(9), 1368–80. doi:10.1097/01.ALC.0000029598.07833.2D

Paul, C. A., Au, R., Fredman, L., Massaro, J. M., Seshadri, S., Decarli, C., & Wolf, P. A. (2008).

    Association of alcohol consumption with brain volume in the Framingham study. *Archives

    of Neurology*, *65*(10), 1363–7. doi:10.1001/archneur.65.10.1363

Perret, E. (1974). The left frontal lobe of man and the suppression of habitual responses in verbal

    categorical behaviour. *Neuropsychologia*, *12*(3), 323–30.

Peterson, B. S., Kane, M. J., Alexander, G. M., Lacadie, C., Skudlarski, P., Leung, H. C., Gore,

    J. C. (2002). An event-related functional MRI study comparing interference effects in the

    Simon and Stroop tasks. *Brain Research. Cognitive Brain Research*, *13*(3), 427–40.

Pfefferbaum, A., Lim, K. O., Zipursky, R. B., Mathalon, D. H., Rosenbloom, M. J., Lane, B.,

    Sullivan, E. V. (1992). Brain gray and white matter volume loss accelerates with aging in

chronic alcoholics: a quantitative MRI study. *Alcoholism, Clinical and Experimental Research*, *16*(6), 1078–89.

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 and Experimental Research*, *21*(3), 521–9.

Pfefferbaum, A., Sullivan, E. V, Mathalon, D. H., Shear, P. K., Rosenbloom, M. J., & Lim, K. O. (1995). Longitudinal changes in magnetic resonance imaging brain volumes in abstinent and relapsed alcoholics. *Alcoholism, Clinical and Experimental Research*, *19*(5), 1177–91.

Roth, R. M., Koven, N. S., Randolph, J. J., Flashman, L. A., Pixley, H. S., Ricketts, S. M., Saykin, A. J. (2006). Functional magnetic resonance imaging of executive control in bipolar disorder. *Neuroreport*, *17*(11), 1085–9. doi:10.1097/01.wnr.0000227979.06013.57

Sager, K.A., Dahlgren, M.K., Gonenc, A., Racine, M.T., Dreman, M.W., & Gruber, S.A. (2016). The impact of initiation: Early onset marijuana smokers demonstrate altered Stroop performance and brain activation. *Developmental Cognitive Neuroscience, 16*, 84-92.

Schweinsburg, A. D., McQueeny, T., Nagel, B. J., Eyler, L. T., & Tapert, S. F. (2010). A preliminary study of functional magnetic resonance imaging response during verbal encoding among adolescent binge drinkers. *Alcohol (Fayetteville, N.Y.)*, *44*(1), 111–7. doi:10.1016/j.alcohol.2009.09.032

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 and Alcohol Dependence*, *79*(2), 201–10. doi:10.1016/j.drugalcdep.2005.01.009

Silveri, M.M., Rogowska, J., McCaffrey, A., Yurgelun-Todd, D.A. (2011). Adolescents at risk for alcohol abuse demonstrate altered frontal lobe activation during Stroop performance. *Alcoholism, Clinical and Experimental Research*, 35(2), 218-228.

Squeglia, L. M., Pulido, C., Wetherill, R. R., Jacobus, J., Brown, G. G., & Tapert, S. F. (2012). Brain response to working memory over three years of adolescence: influence of initiating heavy drinking. *Journal of Studies on Alcohol and Drugs*, *73*(5), 749–60.

Smith, S., & Fein, G. (2011). Persistent but less severe ataxia in long-term versus short-term abstinent alcoholic men and women: a cross-sectional analysis. *Alcoholism, Clinical and Experimental Research*, *35*(12), 2184–92. doi:10.1111/j.1530-0277.2011.01567.x

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology*, *18(6)*, 643-662.

Sullivan, E. V, Harding, A. J., Pentney, R., Dlugos, C., Martin, P. R., Parks, M. H., Pfefferbaum, A. (2003). Disruption of frontocerebellar circuitry and function in alcoholism. *Alcoholism, Clinical and Experimental Research*, *27*(2), 301–9. doi:10.1097/01.ALC.0000052584.05305.98

Tapert, S. F., Brown, G. G., Baratta, M. V, & Brown, S. A. (2004). fMRI BOLD response to alcohol stimuli in alcohol dependent young women. *Addictive Behaviors*, *29*(1), 33–50.

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 and Experimental Research, *25*(2), 236–45.

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

    Psychological Corporation).

Wetherill, R. R., Squeglia, L. M., Yang, T. T., & Tapert, S. F. (2013). A longitudinal

    examination of adolescent response inhibition: neural differences before and after the

    initiation of heavy drinking. *Psychopharmacology*, *230*(4), 663–71. doi:10.1007/s00213-

    013-3198-2

Wignall, N., De Wit, H., & Mello, Nancy K. (2011). Effects of Nicotine on Attention and

    Inhibitory Control in Healthy Nonsmokers. *Experimental and Clinical*

    Psychopharmacology, 19, 183-191.

World Health Organization (WHO). (2007). *WHO Expert Committee on Problems Related to*

    Alcohol Consumption – 2nd Report. Geneva, Switzerland: WHO Press.

Table 1. Environmental and IQ variables for alcohol users and controls.

Variable	Users $n = 17$ Mean (SE)	Controls $n = 11$ Mean (SE)	Results
Family income	31769 (14606)	35179 (18875)	$F(1,28) = 0.25$, $p < 0.62$
Average parental education[*]	2.62 (0.92)	2.72 (0.93)	$F(1,28) = 0.09$, $p < 0.77$
Number of years of schooling	11.23 (1.17)	11.36 (0.50)	$F(1,28) = 0.12$, $p < 0.73$
WAIS full scale IQ	111.00 (13.55)	117.46 (11.92)	$F(1,28) = 1.51$, $p < 0.23$
Connors (conduct problems)	0.34 (0.79)	0.31 (0.83)	$F(1,28) = 0.01$, $p < 0.93$
Connors (learning problems)	0.42 (0.95)	0.28 (1.26)	$F(1,28) = 0.10$, $p < 0.76$
Connors (impulsivity-hyperactivity)	-0.07 (1.22)	0.14 (0.75)	$F(1,28) = 0.25$, $p < 0.62$
Connors (anxiety)	0.18 (1.14)	0.45 (1.28)	$F(1,28) = 0.31$, $p < 0.59$
Connors (psychosomatic problems)	0.59 (1.37)	0.45 (1.50)	$F(1,28) = 0.05$, $p < 0.82$

No significant differences were observed between the groups for any variable.

[*]Education was coded as 1 – did not finish high school, 2- graduated from high school, 3 – graduated from college or university, 4- obtained a post graduate degree

Table 2. Drug exposure based on current alcohol use grouping.

Drug exposure	Users $n = 17$[a] Mean (SE)	Controls $n = 11$ Mean (SE)	Results
Current marijuana (joints/week)	5.16 (10.63)	2.00 (4.26)	$F(1,28) = 0.95$, $p < 0.34$
Current nicotine (cigarettes/day)	3.38 (5.87)	2.13 (3.89)	$F(1,28) = 0.42$, $p < 0.52$
Prenatal alcohol (AA/day)[b]	0.26 (0.29)	0.13 (0.21)	$F(1,28) = 1.75$, $p < 0.20$
Prenatal caffeine (mg/day)	86.71 (100.22)	97.17 (117.09)	$F(1,28) = 0.67$, $p < 0.80$
Prenatal marijuana (joints/week)	2.71 (4.32)	6.78 (15.40)	$F(1,28) = 1.08$, $p < 0.31$
Prenatal nicotine (cigarettes/day)	6.05 (9.22)	7.13 (11.74)	$F(1,28) = 0.08$, $p < 0.78$

[a] Current alcohol user (mean 4.72 [SE 0.73] drinks/week)

[b] Ounces of absolute alcohol per day.

Table 3. Performance data for the two Stroop conditions based on alcohol use.

Performance measure	Users $n = 17$ Mean (SE)	Controls $n = 11$ Mean (SE)	Results
Errors of commission (Animals)	4.12 (3.19)	5.67 (5.69)	$F(4, 23) = 0.21$, $p < 0.93$
Errors of commission (Numbers)	10.65 (8.26)	10.58 (8.66)	$F(4, 23) = 0.13$, $p < 0.97$
Reaction time (Animals)	0.68 (0.07)	0.69 (0.10)	$F(4, 23) = 0.83$, $p < 0.371$
Reaction time (Numbers)	0.75 (0.11)	0.74 (0.10)	$F(4, 23) = 0.60$, $p < 0.66$
Stroop Effect (Incongruent – Congruent)	0.66 (0.06)	0.47 (0.03)	$F(4, 23) = 1.78$, $p < 0.17$
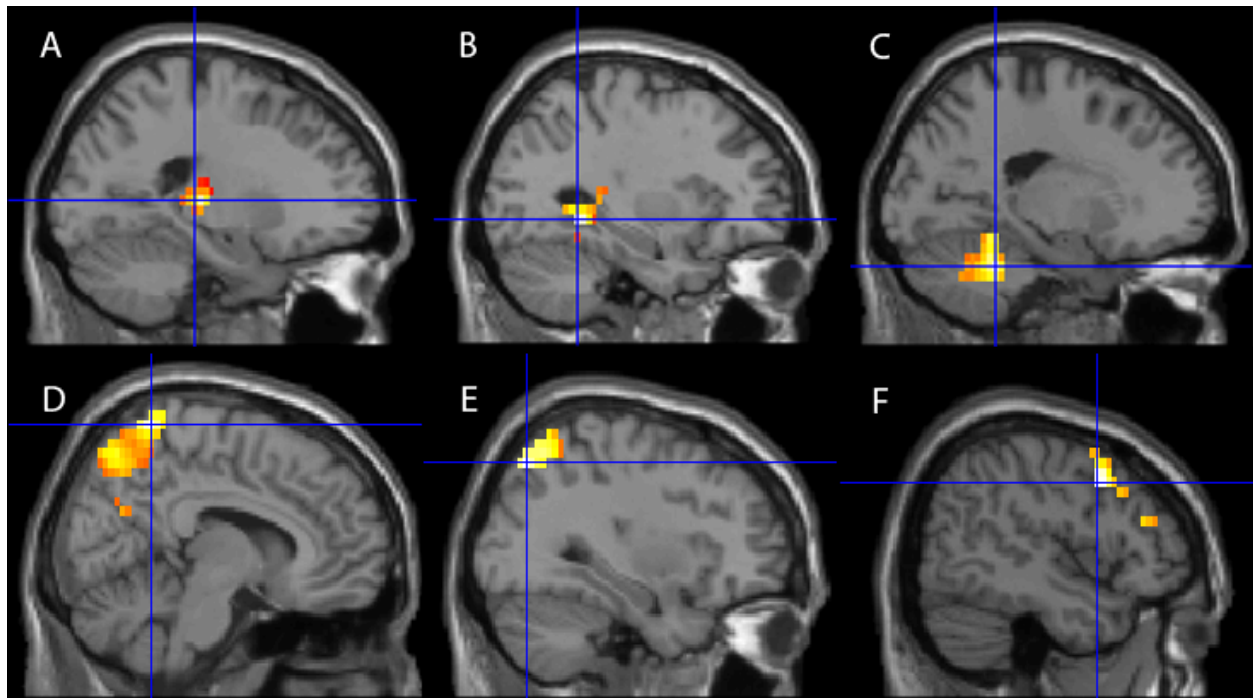
Fig.1. Blue cross hairs highlight areas where there was significantly more activity in current users compared to controls in the 'Numbers minus Animals' condition. Areas of activity are represented as follows: (A) left thalamus (x, y, z = -24 -27 5), (B) left fusiform gyrus (x, y, z = -21 -75 -5), (C) left anterior cerebellum (x, y, z = -12 -45 -25), (D) right precuneus (x, y, z = 9 -51 70), (E) right superior parietal lobe (x, y, z = 9 -51 70) and (F) right precentral gyrus (x, y, z = 45 6 45).

Chapter 3

Neural impact of low-level alcohol use on response inhibition:

An fMRI investigation in young adults

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Abstract

It is widely known that alcohol consumption adversely affects human health, particularly in the immature developing brains of adolescents and young adults, which may also have a long-lasting impact on executive functioning. The present study investigated the neural activity of 28 young adults from the Ottawa Prenatal Prospective Study (OPPS) using functional magnetic resonance imaging (fMRI). The purpose of this study was to discover the impact of regular low-level alcohol consumption on response inhibition as the participants performed a Go/No-Go task. Results indicated that, despite a lack of performance differences, young adults who use alcohol on a regular basis differ significantly from those who do not use alcohol regularly (if at all) with respect to their neural activity as the circuitry engaged in response inhibition is being challenged. Specifically, areas that showed significantly more activation in users compared to controls included the left hippocampus, parahippocampal gyrus, superior frontal gyrus, precentral gyrus, right superior parietal lobule, and the cerebellum. These results suggest that even in low amounts, regular consumption of alcohol may have a significant impact on neurophysiological functioning during response inhibition in the developing brain of youth.

Introduction

While alcohol consumption is widely known to have adverse effects on human health (Feldstein et al., 2014; Petit et al., 2013), research is still in the early stages of understanding the full extent of its detrimental impact on developmental and functional processes in the brain. For example, alcoholism has consistently been shown to cause significant decreases in both grey and white matter volumes, as well as increases in sulcal and ventricular volumes, with the amount of loss accelerated with age (Pfefferbaum et al., 1992; Pfefferbaum et al., 1997; Verbaten, 2009). Chronic alcohol use may also preferentially impact the integrity of frontal white matter tracts, which are particularly important in executive functioning (Fortier et al. 2014; Pfefferbaum et al., 2009).

Importantly, it is now well established that the impact of alcohol use differs among adolescents and adults, with younger individuals being particularly vulnerable to the deleterious effects of alcohol on the developing brain (Guerri and Pascual, 2010; Squeglia et al., 2012; Welch, Carson, & Lawrie, 2013). However, the majority of research in adolescents has focused on the functional consequences of chronic, heavy alcohol use, with little attention focused on the impact of regular light to moderate drinking on the developing brain. This is particularly concerning, as evidence suggests that alcohol related structural brain damage accumulates along a dosage continuum (Paul et al., 2008), highlighting a gap in our current understanding and a need for further research in this area.

Adolescence is a critical period in neurocognitive development, with the brain still undergoing several key neuromaturational processes (Pokhrel et al., 2013). This includes maturation of the prefrontal cortex (PFC), the cortical area responsible for our highest order cognitive processes, which continues developing until the latter part of young adulthood (Lopez-

Caneda et al., 2014). One of the hallmarks of executive control required for day-to-day functioning is response inhibition, which refers to the ability to withhold inappropriate responses or interrupt a cued response (Tamm et al., 2002). This may interfere with goal-directed behaviours when presented with new, corrective information (Crews et al., 2009; Smith et al., 2011).

Poor response inhibition has been shown to be predictive of impulsivity and sensation seeking (Castellanos-Ryan, Rubia & Conrod, 2011), which is particularly concerning when considering the possibility of future substance dependence, as this may be a critical factor in being able to achieve and maintain subsequent abstinence (Pokhrel et al., 2013). The results of the Fortier et al. (2014) study showed that neural areas most impacted by alcohol are those that underlie inhibitory control in the brain, including frontostriatal circuits, superior and cerebellar tracts. In light of this and the importance of response inhibition in our every day functioning, understanding how alcohol impacts this aspect of executive functioning in the developing brain warrants investigation. Given that other parts of the world have different legal drinking ages and the suggestion that underage illegal drinking may be more strongly associated with impulsivity than legal drinking, it is important to mention that all participants in the current study were of legal drinking age for this province/country.

The Go/No-Go task is a well-established fMRI compatible measure of response inhibition in which participants are initially required to respond to a stimulus, thus creating a strong cued tendency to respond, which is followed by withholding response to the conditioned stimulus (Wright et al., 2014). In healthy adult controls, the Go/No-Go task has been shown to be dependent on activation of the anterior cingulate, dorsolateral prefrontal cortex, inferior frontal

cortex, inferior parietal lobule, premotor cortex, thalamus, and the caudate using fMRI

(Anderson et al., 2011; Longo et al., 2013; Stevens et al., 2007; Stevens et al., 2009).

Norman et al. (2011) conducted a longitudinal study using the Go/No-Go task to

investigate whether abnormalities in response inhibition was predictive of heavy alcohol use in

an adolescent sample. Participants were scanned using fMRI at baseline only. Although there

were no performance differences across groups, those who later transitioned to heavy alcohol use

showed significantly less activity in a number of brain regions compared to those who did not go

on to use heavily. This included less activation of the right inferior frontal gyrus, left dorsal and

medial frontal areas, bilateral motor cortex, cingulate gyrus, left putamen, bilateral middle

temporal gyri, and bilateral inferior parietal lobules. These findings indicated there may be

important pre-existing neural vulnerabilities in the ability to adequately impose restraint on risk-

taking behaviours, such as substance abuse, and this may be exacerbated in more complex

situations requiring more difficult decision-making.

Admadi et al. (2013) compared light and heavy alcohol users using a Go/No-Go task and

fMRI. Results indicated that light drinkers had greater functional activation in the left superior

motor area, bilateral parietal lobe, right hippocampus, bilateral middle frontal gyrus, left superior

temporal gyrus, and cingulate gyrus relative to heavy users. Moreover, heavy users also showed

performance deficits during the task having significantly slower reaction times for both

experimental and control trials. These results reflect that heavy drinking has a pervasive

depressant effect on the neural areas associated with response inhibition, as well as actual ability

to suppress cued responses during task performance. In support of the Norman et al. (2011)

results, these findings may translate to real-life difficulties with overcoming impulsive

tendencies that ultimately lead to substance abuse and poor behavioural outcomes.

In contrast, Ames et al. (2014) conducted another fMRI study comparing young adult light and heavy alcohol users on the Go/No-Go task using alcohol cues. They found that heavy users showed significantly more functional activity in the right dorsolateral prefrontal cortex, medial frontal cortex, cingulate, and insula. Heavy drinkers also had impaired performance, with faster response rates and fewer correct responses compared to light users. These results reflect that heavy alcohol use results in impulsive responding leading to performance deficits. It is also possible that the regions associated with task performance are working harder, as well as areas such as the hippocampus not typically associated with the Go/No-Go, in order to complete the task.

Given the disparity of results and that the majority of research on the effects of alcohol on response inhibition in young adults has consisted of comparisons of light to heavy drinkers or non-drinkers to heavy drinkers, further research on the functional consequences of lower levels of regular alcohol use in adolescence is warranted. Alcohol use, particularly in young adults, is also commonly associated with the use of other illicit drugs (WHO, 2007) and, as such, it is essential to control for these confounds in neuroimaging investigations to have a true understanding of how alcohol impacts brain functioning. Although there are currently few fMRI studies that have examined the impact of adolescent alcohol use on neurocognitive development and functioning (Feldstein et al., 2014; Petit et al., 2013; Squeglia et al., 2012, etc.), with even fewer studies that have controlled for additional illicit substances, further study is needed.

To account for this, the present study uses data collected from the Ottawa Prenatal Prospective Study (OPPS), a longitudinal investigation that has followed a cohort of individuals from in utero for over 20 years, acting as a source of rich information on potentially confounding lifestyle and drug exposure variables. Unlike many other samples used in drug research, this

unique group of individuals allows us to investigate the neurobehavioural effects of alcohol use on executive functioning while controlling for other prenatal and adolescent/young adult drug exposures, as well as other lifestyle variables such as education or socioeconomic status.

In a previous study conducted in our laboratory using the OPPS sample, Hatchard et al. (2015) used the Counting Stroop, a task that measures cognitive interference, to study the effects of low-level alcohol use on this aspect of executive functioning. Our results showed that, despite a lack of performance difference between low-level alcohol users and controls, regular use was correlated with a significant change in neural activity. This included greater functional activation of the cerebellum, thalamus, fusiform gyrus, prefrontal cortex, and precuneus. It is noteworthy that many of these regions are not typically activated during performance of the Counting Stroop. This may reflect that, despite using small amounts of alcohol, the brains of users were engaging in compensatory strategies through recruitment of additional regions and this may possibly underlie deficits within the attention circuitry. These findings are paralleled with previous studies that have also found that alcohol users show the recruitment of additional brain regions, possibly to compensate for cognitive demands during task performance (Sullivan, Harris, & Pfefferbaum, 2010; Wetherill et al., 2013).

Using the same sample of participants recruited from the OPPS for the Counting Stroop study, the present study used fMRI during a Go/No-Go task, to investigate the impact of adolescent onset low-level alcohol use on response inhibition in young adulthood. It was predicted that no performance differences would be observed between alcohol users and controls while completing the task. However, based on the areas of the brain required for response inhibition and the critical changes that occur in the PFC during this period of neurocognitive development, when the onset of alcohol use often begins, neurophysiological differences were

anticipated between groups. It was hypothesized that regular alcohol consumption would

negatively impact areas of the brain related to response inhibition, including the prefrontal cortex

and cerebellum. It was anticipated that this would be observed as increased activation and that it

would be accompanied by increased activity in other regions, not typically associated with

performance of a Go/No-Go task, namely the hippocampus.

Methods

Participants

Participants consisted of a random sample of 34 individuals recruited from the OPPS. All

participants provided informed consent and were imaged regardless of sex or drug exposures.

The inclusion criteria for participation required that participants were at least 19 years old (legal

drinking age in Ontario), right-handed, native English speakers, and had recently completed a

comprehensive neuropsychological assessment. Participants were also required to meet fMRI

compatibility criteria, which included no metal implants, no pacemaker, no recent surgery,

suitable or corrected vision for viewing stimuli, and no previous metal in their eyes. Also,

participants who had acquired a head injury with a loss of consciousness were not included in the

study. Participants were excluded from the present study if they tested positive for

amphetamines, opiates, cocaine, crack, heroin, lysergic acid diethylamide, solvents, steroids,

and/or mushrooms during urinalysis or self-reported regular use of any of these drugs (once or

more per month; 4 participants), had a history of an Axis I diagnosis based on the DSM-IV-TR

(1 participant), or had any structural abnormalities detected during the MRI scan (1 participant).

The purpose of the investigation was to determine if consumption of low, but

regular, amounts of alcohol has an impact on the neurophysiology of response inhibition relative

to individuals who sporadically consume alcohol, if at all. As such, a cut-off of >1 drink per

week, for more than a year, was used to determine the alcohol users group. Participants who

consumed <1 drink/week and <5 drinks/month, on average, were assigned to the control group.

Based on these criteria, 17 alcohol users (9 male, 8 female; mean age = 20.06 [SE=0.23]; range

19-21 years old) and 11 controls (5 male, 6 female; mean age = 20.09 [SE=0.25]; range 19-21

years old) were included in the present analyses. Alcohol use was based on the number of

alcoholic drinks consumed per week, with users reporting an average of 4.72 drinks/week (SE =

0.74), 1 to 2 times/week, over the previous year, with an average of 14.3 years for the mean age

of first exposure (ranging from ages 12 to 16). Only sporadic alcohol consumption, if any, was

reported within the controls group; 3 participants did not drink at all and the other 9 reported an

average of <4 drinks/month, with a mean age of 15.5 years for first alcohol exposure (ranging

from ages 13 to 19). Among alcohol users, 7 participants had used nicotine within their lifetime,

with 6 being current nicotine users, whereas within the control group there were 4 lifetime

nicotine users, with 3 of them being current nicotine users.

Clinical and drug use assessments

Both psychological and neuropsychological assessments had been performed within 1

year of imaging. This included the Wechsler Adult Intelligence Scale-III (WAIS-III) (Wechsler,

1997) and the Computerized Diagnostic Interview Schedule for Children (Bacon, 1997), used to

assess current mental health conditions based on DSM-IV-TR criteria. Parents of participants

were asked to complete the Conners' Parent Rating Scale (Goyette, Conners, & Ulrich, 1978)

and provide information related to socioeconomic status (i.e. parental education and family

income). There were no significant differences found between current alcohol users and controls

on any of these measures or in terms of socioeconomic status. As a result, they were not included

in the analyses (Table 1). In order to control for current drug use, participants were asked to provide a urine sample and complete a self-report drug questionnaire upon arrival for MRI scanning. Specifically, participants were screened for cannabis, amphetamines, opiates, cocaine, nicotine (with cotinine), crack, heroin, lysergic acid diethylamide, solvents, steroids, and mushrooms. The samples were tested for creatinine as an indicator of urine dilution.

Imaging protocol

Data acquisition. All participants were imaged on a 1.5 Tesla Siemens Magnetom Symphony MR scanner. Participants were required to lay supine, with their heads secured in a custom head holder while a conventional T1-weighted spin echo localizer was acquired and used to prescribe a subsequent 3D FLASH (TR/TE 11.2/21 ms, flip angle 60°, field of view (FOV) 26 × 26 cm, 256 × 256 matrix, slice thickness 1.5 mm) volume acquisition. Whole-brain blood oxygen level-dependent (BOLD) fMRI was performed using a T2*-weighted echo planar pulse sequence (TR/TE 3000/40 ms, flip angle 90°, FOV 24 × 24 cm, 64 × 64 matrix, slice thickness 5 mm, 27 axial slices, bandwidth 62.5 kHz).

Equipment. Participants were presented with stimuli as white letters on a black background on a screen located at the entrance of the magnet, which was viewed through a mirror mounted on the head coil. Participants provided responses using an MRI-compatible fiber optic response pad (Lightwave Medical, Vancouver, British Columbia, Canada). The Visual and Auditory Presentation Package (VAPP) was used to present and record responses to stimuli. All lighting was turned off during the imaging.

Go/No-Go task. Participants were required to press as quickly and correctly as possible with their right index finger. If they made a mistake, they were instructed to continue without

thinking about the mistake. The scanning session started with an initial resting epoch of 9-sec, which allowed longitudinal magnetic relaxation (T1 effects) to stabilize. The images that were collected over this initial rest epoch were not included in the data analysis.

The task was conducted as a block design, which involved presentation of letters one at a time in the middle of the screen for a period of 75 ms and included an interstimulus interval of 925 ms. Fifty percent of the stimuli were 'X' and the other 50% were other capital letters selected randomly from the remainder of the alphabet, which has been previously shown to consistently activate the "Go bias" of response inhibition (Donkers & van Boxtel, 2004; Menon et al., 2001; Shenoy & Yu, 2012). All letters were presented in a random order but ensured that the 'X' was presented 50% of the time, building up a prepotent response to 'X'. All letters were presented in a random order. The task included two conditions. In the control condition, "Press for X", participants were instructed to press a button using their right index finger whenever an X appeared on the screen and refrain from pressing for all other letters. During the experimental condition, "Press for all letters except X", participants were required to refrain from pressing for X and rather press for all other letters (see Appendix A, Figure 2 for diagram of the task).

Prior to imaging, participants performed a practice session of 10 trials of each condition of the Go/No-Go task outside of the scanning room. During the scanning sessions, there were 4 experimental blocks and 4 control blocks, with 24 stimuli in each block. The session began with two 'Press for X' control blocks to ensure the prepotent response to the 'X'. Both conditions were presented in epochs of 27-sec duration, with each epoch preceded by a 3-sec instruction epoch, followed by a 24-sec rest epoch. In the instructional epochs, participants were directed to "Press for X" or "Press for all letters except X". In the rest epochs, "REST" was presented on the screen and participants were not required to make any motor responses. This task design has

been successfully used previously in several populations (Longo et al., 2013; Scherling et al.,

2012; Smith et al., 2011, 2009).

Performance parameters and analyses. Errors of commission were recorded for any

response following a No-Go stimulus (i.e. pressing stimulus 'T' for the 'Press for X' condition or

pressing X for the 'Press for all letters except X' condition) within 900 ms of stimulus

presentation. Errors of omission were recorded each time a participant failed to respond to the

target stimulus within the allotted time frame. Separate ANCOVAS were performed on reaction

time data, errors of commission, and errors of omission using current nicotine, current marijuana,

and prenatal alcohol as covariates, which were selected given that these variables have been

shown to impact performance on inhibitory responding tasks, such as the Go/No-Go (Burden et

al., 2010; Ettinger et al., 2017; Hatchard et al., 2015; Luijten, Little, & Franken, 2011; Smith et

al., 2011; Wrege et al., 2014; Zhao et al., 2016). As such, the use of the selected covariates adds

another level of certainty that the results are based on the unique contribution of alcohol to

performance.

Image post-processing. Using Statistical Parametric Mapping (SPM8) software,

functional brain images were realigned to correct for motion by employing the procedure of

Friston et al. (1995). For all participants, the motion correction did not exceed 1 mm. This was

followed by spatially normalizing images to match the echo planar imaging (EPI) template

provided in SPM8. All images were subsequently smoothed with an 8-mm full-width at half-

maximum Gaussian filter. To remove frequency components below 0.36 Hz, the observed time

course of image intensity within each voxel was temporally filtered and, using the general linear

model, fitted to a hemodynamic response model consisting of sequential contributions from

sequential epochs. For each type of epoch, the hemodynamic response was convolved with a 6-

sec filtered boxcar waveform and subjected to the same high-pass temporal filter as the observed time course.

Whole brain analyses with SPM8. Separate first-level fixed effects analyses were performed on all participants individually and then statistical parametric maps were obtained for the two groups: current alcohol users and controls. Contrast images were calculated for these analyses using the comparison of 'Press for all letters except X' – 'Press for X'. These contrast images were subsequently used for the second-level random-effects analyses, which allowed for a comparison between alcohol users and controls.

Using a set threshold of $p_{uncorr} = 0.001$, multiple independent samples t-tests were performed. A cluster-wise correction was performed at $p_{FWE} = 0.05$, which allowed for the assessment of differences in neural activity between users and controls during the 'Press for all letters except X' – 'Press for X' contrast, while manipulating the covariates included in the analysis. The reported results are those comparing current alcohol users and controls using prenatal alcohol, current marijuana, and current nicotine as covariates. Although these variables were not significantly different between groups, given the small sample size and previous literature suggesting an impact of these drugs on response inhibition, they were used as covariates in the present analyses. This was an attempt to ensure that observed results were not attributable to any other prenatal or current drug effects.

Results

Participant characteristics

Weekly current alcohol use was significantly different between the two groups ($F = 33.5$; $p<0.001$). Notably, there were no significant differences observed between users and controls based on other drug exposures (Table 2). No participants from either group reported alcohol

consumption on the day of testing, which eliminates the possibility that the results represent

acute alcohol effects rather than the effects of regular consumption on neural processing. Four

alcohol users and 2 controls regularly used marijuana, defined as at least 1 joint/day.

Additionally, 5 alcohol users and 4 of the controls reported nicotine use on a regular basis (i.e.

smoking every day). There was no other illicit drug use self-reported or observed in urinalysis.

All analyses were performed with and without these individuals to eliminate current nicotine or

marijuana use in order to eliminate these as possibilities for the results obtained. There were no

differences observed between these analyses and, as such, only the results that included all

participants are reported below.

Drug questionnaire and urine sample data

To control for urine dilution, all metabolite concentrations were adjusted for creatinine.

For the validity of current use, the quantity of drug used over the weeks prior to testing and over

the past month was compared. The Pearson correlation between the urine samples for cannabis

levels and the drug questionnaire was 0.97 (p<0.001) and nicotine (cotinine/creatinine) was 0.91

(p<0.001). In light of the high correspondence, this validated the use of the drug questionnaire

for current drug use and user history.

Performance data

There were no significant differences found between current alcohol users and controls

for errors of commission, errors of omission, reaction time for the 'Press for X', or reaction time

for 'Press for all letters except X" based on the ANCOVA results (see Table 3).

fMRI data

The within-group, first-level fixed-effects analyses showed an expected pattern of

activity for both groups, which included increased neural activity for the 'Press for all letters

except X' – 'Press for X' contrast in the dorsolateral prefrontal cortex (DLPFC). Despite this similar pattern of anticipated brain activity across groups, significant differences were revealed when performing the second-level random-effects comparison.

The most prominent results are presented at threshold $p_{crit} < 0.001$ uncorrected with $p_{FWE} = 0.05$ cluster-wise correction (see Figure 1). Current alcohol users showed significantly increased neural activity compared to controls in the left superior frontal gyrus (x, y, z = -30, 51, 30) and the left precentral gyrus (x, y, z = -39, -18, 65), which made up a cluster of 1115 voxels (p<0.001). In a second cluster of 595 voxels (p<0.005), there was significantly more brain activation in the right superior parietal lobule (x, y, z = 18, -51, 75) and the right cerebellum (x, y, z = 15, -39, -20). Lastly, in a third cluster of 248 voxels (p<0.05), alcohol users showed significantly more activation than controls in the left hippocampus (x, y, z = -30, -36, -5) and parahippocampal gyrus (x, y, z = -24, -54, 0).

Supplemental analyses were performed excluding the participants who smoked marijuana on the day of testing (n = 3, no participant smoked more than 1 joint or within 6-hr of scanning). No differences were observed in the results with or without these participants, which suggests that the results obtained were not directly attributable to acute effects of marijuana use.

Discussion

The current study compared the fMRI BOLD response on a Go/No-Go task in young adults that regularly consumed low-levels of alcohol with individuals who did not drink regularly, if at all. Although there were no differences observed in performance on this task of response inhibition, there were differences in regional brain activation between alcohol users and

controls when challenged by the inhibitory processing of the task. Despite only regular

consumption of low amounts of alcohol, compared to controls these individuals showed an

extensive pattern of increased neural activity in the superior frontal gyrus, precentral gyrus,

hippocampus, parahippocampal gyrus, cerebellum, and superior parietal lobe.

The most prominent differences between groups were observed in the left superior frontal

gyrus and precentral gyrus, regions typically involved in response competition and the

preparatory process leading to correct initiation or suppression of movement (Yamaguchi et al.,

2008). There were no differences in performance to account for this difference and thus the

increased activity in these regions during response inhibition could be interpreted as a need to

work harder in alcohol users compared to controls. This is a common interpretation of fMRI

results of increased brain activity (Gevins and Smith, 2000) and highlights the sensitivity of

fMRI to provide valuable information about differences in brain activity that could not be

identified with performance data alone. There could be other interpretations of the results,

however, confirmation is not possible without further investigation. Most fMRI results of

increased activity with similar performance is taken to reflect compensatory mechanisms.

Recruitment of different pathways, a reallocation of neural resources, all suggests some form of

reorganization of neurophysiological mechanisms to successfully perform the task (Han et al.,

2009). This explanation is also supported by the increased activity in the superior parietal lobe of

the users compared to controls. The Go/No-Go task typically activates the superior parietal lobe

for regulation and self-monitoring of responses. This processing appears to be influenced by

regular alcohol consumption in the young adult.

The cerebellum is another region necessary for completion of the Go/No-Go task, given the motor component within the task itself and this regions role in motor functioning. Similarly, as this region was significantly more active compared to controls, it may indicate the brain is working harder to complete the task adequately. The pattern of activity observed in the superior frontal gyrus and the cerebellum may be explained by alcohol related changes to the fronto-cerebellar pathway. These connections are thought to underlie higher order cognitive functioning and have been shown to be negatively impacted by chronic alcohol use. This may underlie the cognitive deficits observed in chronic alcoholics. In particular, alcohol abuse causes dissociation within this brain circuit as communication between frontal areas and the cerebellum is interrupted. Using fMRI, Rogers et al. (2012) compared recently abstinent alcoholics to healthy controls on a finger-tapping task, where participants were instructed to tap in response to visual cues. Their results showed that alcohol dependent adults had a significant reduction in fronto-cerebellar connectivity compared to controls, which may be the result of neuronal injury within these regions as a result of alcoholism. This damage may underlie some of the common neuropsychological deficits associated with alcohol-induced neural injury and may be particularly vulnerable within the developing brain. In another study, Herting, Fair, and Nagel (2011) examined fronto-cerebellar connectivity in a sample of at-risk alcohol naïve youth based on familial history of alcoholism. Compared to controls, the at-risk group had lower white matter integrity within the regions associated with the fronto-cerebellar circuit, as well as reduced functional connectivity in this network based on completion of number of tasks (e.g. Counting Stroop, reward based decision making task, etc). It would be of interest to perform diffusion tensor imaging (DTI) in the OPPS sample to investigate further the connectivity within the white matter tracts.

In addition to the characteristic regions activated during response inhibition, the current study also found recruitment of additional brain areas that are not typically involved in performing the Go/No-Go task. This included the left hippocampus and parahippocampal gyrus. These areas of the brain are located in the medial temporal lobe and have a specific role in memory processing. Although there is a working memory component involved in task completion (i.e. remembering when to Press for X or Press for all letters except X), significant activation of these areas is possibly attributable to these resources being strained. Again, this could potentially reflect compensation recruitment of additional areas in order to be able to complete the task. Alternatively, it could also point to different strategies for performing the task. The hippocampus has been shown to be both vulnerable and negatively impacted by chronic alcohol use. Previous research has found that chronic heavy alcohol use causes significant reductions in hippocampal volume, which in turn, contributes to impairments in memory and cognitive difficulties (Beresford et al., 2006). The hippocampus may be particularly vulnerable during young adulthood. These results present a potential precursor of further detrimental changes that may occur, even with low amounts of chronic alcohol use, if use continues. Further research could help elucidate exactly how alcohol influences hippocampal function over time and whether this occurs across the alcohol dosage continuum.

The findings of this study support and parallel the results of Hatchard et al. (2015), which examined how low-level alcohol consumption impedes cognitive interference. In that study, participants from the OPPS completed the Counting Stroop task, which is a commonly used measure of cognitive interference. Similar to the current study, results showed no performance differences between groups but increased neural activity was observed in several regions in current alcohol users compared to controls. This included significantly more activity in the

fusiform gyrus, superior parietal lobule, precuneus, and cerebellum, as well as the differences in

the prefrontal cortex, cingulate gyrus, and superior temporal gyrus. Importantly, many of these

regions are not typically involved in neural processing during Counting Stroop performance.

Both studies are suggestive of different neurophysiological recruitment among regular

alcohol users to complete the tasks. Should further levels of difficulty be added to either task, the

attentional circuitry required for adequate performance may not be able to adequately

compensate for the neural changes observed across current users. This may also occur in real-

world situations, such as daily work activities, which would require more sustained attention.

Further studies using the OPPS, perhaps using more complex tasks, could be beneficial in further

isolating the impact that adolescent onset alcohol use has on attention and concentration. This

would also help clarify whether the differences in neurophysiology are related to potential future

deficits in response inhibition that could not be targeted with such a basic Go/No-Go task or

simply differences in information processing and strategy to perform the task. It would also be

valuable to conduct a follow-up study using the same participants, as this would provide insight

into whether the differences observed between users and controls are long-lasting, as well as

whether they become more detrimental in cognitive functioning over time.

Our findings are consistent with those of Ames et al. (2014), which found that light

drinking in young adults showed patterns of neural activity suggestive of compensatory

recruitment while performing the Go/No-Go. However, there were a number of differences in the

regions showing significantly more activity. While both studies found more activity in frontal,

parietal, and hippocampal regions, their study also showed activation in the superior motor area,

superior temporal gyrus, and cingulate, whereas our study found more activity in the precentral

gyrus, cerebellum, and parahippocampal gyrus. One of the reasons for these differences may be attributable to differences in the samples used, as the current study compared irregular/no use controls to low-level users, whereas their study compared low-level drinkers to heavy drinkers. Moreover, the Ames et al. study did not control for additional substance use in their analysis, which may also help explain some of the differences observed. Despite these differences, the overall findings across both studies are similar in that both are suggestive of altered brain activity to complete the task in youth using alcohol.

Limitations

Importantly, however, it is necessary to consider that the reason and interpretation for differences observed in neurophysiology between groups during the Go/No-Go task remains unclear. While it is possible that the neurophysiological differences observed in the present results are related to compensatory recruitment, this is simply a hypothesis of what may be occurring and other possibilities remain. For example, it may be the case that the differences in regional brain activation between alcohol users and controls are simply reflective of differences in processing information while engaging in response inhibition as opposed to potential neural impairments. Similarly, it could be a result of different neural strategies being implemented between groups. Moreover, due to the lack of research on how low to moderate drinking impacts neurophysiology and response inhibition, much of the comparison made in the present study to previous research relied on research conducted in heavy drinkers. This highlights a gap in the current research literature and further research on regular low alcohol consumption is required to elucidate whether the hypothesis of compensatory recruitment presented in the current findings can explain differences in neural activation.

The present study has a number of other limitations that are also important to consider. For example, the sample size was relatively small and primarily a Caucasian, middle-class population. As a result, replication of these findings is essential, as well as recruiting more diverse ethnic and socioeconomic populations in order to further understand similarities and differences across low and high-risk populations. Similarly, the disproportionate sample size of the groups should be mentioned. This was a result of the random collection of data ie. participants were contacted and imaged until the financial limit for scanner time was met. This small sample size also makes the comparison of results with and without the marijuana/nicotine users included less informative and requiring further study. In light of the low quality of structural scans acquired during imaging, it was not possible to assess whether differences in activation between users and controls could be related to structural differences. In addition, the experimental paradigm used was a block design rather than an event related design. One of the limitations of using block designs is the inability to separate the Go from the No-Go trials. As such, an event-related design may have been useful in separating response inhibition from other cognitive processes.

Conclusion

The results of the current study show that regular consumption of alcohol, even in low amounts, has an impact on neurophysiology in the developing brain. The validity of these results is strengthened by the use of the unique OPPS sample that permitted control over important lifestyle variables, such as drug exposure across the lifespan. This further allows for the current results to elucidate the selective contributions of alcohol on neural processing during response inhibition. Consequently, the present study provides preliminary insight into the potential vulnerability of the developing brain to alcohol. In particular, these findings increase our

understanding that alcohol use in young adulthood changes neurophysiology during response inhibition when performing a Go/No-Go task.

Despite a lack of performance deficits when performing this task of response inhibition, there was significantly increased neural activity in several brain regions in alcohol users compared to controls. This may reflect the brain recruiting additional brain regions to compensate for possible underlying deficits that require increased cognitive demand. However, this interpretation remains unclear and further research is required to clarify whether the differences observed between groups is related to neural compensation or simply differences in information processing and strategic approach to the task. Nevertheless, these results show that even in low amounts, alcohol has a significant impact on neurophysiology in the developing brain of youth.

References

Ahmadi, A., Pearlson, G.D., Meda, S.A., Dager, A., Potenze, M.N., Rosen, R., Austad, C.S.,

Raskin, S.A., Fallahi, C.R., Tennen, H., Wood, R.M., & Stevens, M.C. (2013). Influence

of alcohol use on neural response to Go/No-Go task in college drinkers.

Neuropsychopharmacology, 38, 2197-2208.

Ames, S.L., Wong, S.W., Bechara, A., Cappelli, C., Dust, M., Grenard, J.L., & Stacy, A.W.

(2014). Neural correlates of a Go/No-Go task with alcohol stimuli in light and heavy

drinkers. Behavioural Brain Research, 274, 382-389.

Anderson, B.M., Stevens, M.C., Meda, S.A., Jordan, K., Calhoun, V.D., & Pearlson, G.D.

(2011). Functional imaging of cognitive control during acute alcohol intoxication.

Alcoholism: Clinical and Experimental Research, 35, 156-165.

Bacon, W. (1997). *NIMH-Computerized Diagnostic Interview Schedule for Children-version IV*

(C-DISC 4). (New York, NY: Columbia University).

Beresford, T.P., Arciniegas, D.B., Alfers, J., Clapp, L., Martin, B., Du, Y., Liu, D., Shen, D., &

Davatzikos, C. (2006). Hippocampus volume loss due to chronic heavy drinking.

Alcoholism: clinical and experimental research, 30, 1866-1870.

Burden, M.J., Jaconson, J.L., Westerlund, A., Lundahl, L.H., Morrison, A., Dodge, N.C.,

Klorman, R., Nelson, C.A., Aison, M.J., & Jacobson, S.W. (2010). An event-related

potential study of response inhibition in ADHD with and without prenatal alcohol

exposure. *Alcoholism: Clinical and Experimental Research, 34,* 617-627.

Castellanos-Ryan, N., Rubia, K., & Conrod, P.J. (2011). Response inhibition and reward

response bias mediate the predictive relationships between impulsivity and sensation

seeking and common and unique variance in conduct disorder and substance misuse.

Alcoholism: Clinical and Experimental Research, 35, 140-155.

Crews, F.T., & Boettiger, C.A., (2009). Impulsivity, frontal lobes, and risk for addiction.

Pharmacology, Biochemistry, and Behaviour, 93, 237-247.

Donkers, F.C.L., & van Boxtel, G.R.M. (2004). The N2 in go/no-go tasks reflects conflict

monitoring not response inhibition. *Brain and Cognition, 56,* 165-176.

Ettinger, U., Faiola, E., Kasparbauer, A.M., Petrovsky, N., Chan, R.C.K., Liepelt, R., & Kumari,

V. (2017). Effects of nicotine on response inhibition and interference control.

Psychopharmacology, DOI: 10.1007/s00213-017-4542-8.

Feldstein Ewing, S. W., Sakhardande, A., & Blakemore, S. (2014). The effect of alcohol

consumption on the adolescent brain: A systematic review of MRI and fMRI studies of

alcohol-using youth. *NeuroImage: Clinical*, *5*, 420-437.

Friston, K.J., Ashburner, J., Poline, J.B., Frith, C.D., Heather, J.D., Frackowiak, R.S.J.. (1995).

Spatial realignment and normalization of images. *Human Brain Mapping*, *2*, 165–89.

Frontier, C.B., Leritz, E.C., Salat, D.H., Lindemer, E., Maksimovskiy, A.L., Shepel, J., Williams,

V., Venne, J.R., Millberg, W.P., & McGlinchey, R.E. (2014). Widespread effects of

alcohol on white matter microstructure. Alcoholism: Clinical and Experimental Research,

38, 2925-2933.

Gevins, A., Smith, M.E. (2000). Neurophysiological measures of working memory and

individual differences in cognitive ability and cognitive style. *Cerebral Cortex*, *10*, 829-

839.

Goyette, C. H., Conners, C. K., & Ulrich, R. F. (1978). Normative data on revised Conners

      Parent and Teacher Rating Scales. *Journal of Abnormal Child Psychology*, *6*(2), 221–36.

Guerri, C., & Pascual, M. (2010). Mechanisms involved in the neurotoxic, cognitive, and

      neurobehavioral effects of alcohol consumption during adolescence. *Alcohol*, *44*(1), 15–

      26. doi:10.1016/j.alcohol.2009.10.003

Han, D., Bangen, K.J., Bondi, M.W. (2009). Functional magnetic resonance imaging of

      compensatory neural recruitment in aging and risk for Alzheimer's disease: Review and

      recommendations. *Dementia and Geriatric Cognitive Disorders*, *27*, 1-10.

Hatchard, T., Smith, A.M., Halchuk, R.E., Longo, C., Fried, P.A., Hogan, M.J., & Cameron, I.

      (2015). Effects of low-level alcohol use on cognitive interference: An fMRI study in

      young adults. *Alcohol*, *49*, 7-13. DOI: 10.1016/j.alcohol.2014.07.020.

Herting, M. M., Fair, D., & Nagel, B. J. (2011). Altered fronto-cerebellar connectivity in

      alcohol-naïve youth with a family history of alcoholism. *NeuroImage*, *54*(4), 2582–9.

      doi:10.1016/j.neuroimage.2010.10.030.

Longo, C.A., Fried, P.A., Cameron, I., Smith, A.M. (2013). The long term effects of prenatal

      nicotine exposure on response inhibition: An fMRI study of young adults.

      Neurotoxicology and Teratology, 39C, 9-18.

Menon, V., Adleman, N.E., White, C.D., Glover, G.H., & Reiss, A.L. (2001). Error-related brain

      activation during a Go/NoGo response inhibition task. *Human Brain Mapping, 12,* 131-

      143.

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.

Drugs and Alcohol Dependence, *119*, 216-223.

Lopez-Caneda, E., Holguin, S.R., Corral, F.C., & Doallo, S. (2013). Impact of alcohol use on

inhibitory control (and vice versa) during adolescence and young adulthood: A review.

Alcohol and Alcoholism, 49, 173-181.

Luijten, M., Littel, M., & Franken, I.H.A. (2011). Deficits in inhibitory control in smokers

during a Go/NoGo task: An investigation using event-related brain potentials. *PLoS One,

6,* e18898.

Petit, G., Maurage, P., Kornreich, C., Verbanck, P., & Campanella, S. (2013). Binge drinking in

adolescents: A review of neurophysiological and neuroimaging research. *Alcohol and

Alcoholism*, *49*(2), 198-206. doi:10.1093/alcalc/agt172

Pfefferbaum, A., Lim, K. O., Zipursky, R. B., Mathalon, D. H., Rosenbloom, M. J., Lane, B.,

Sullivan, E. V. (1992). Brain gray and white matter volume loss accelerates with aging in

chronic alcoholics: a quantitative MRI study. *Alcoholism, Clinical and Experimental

Research*, *16*, 1078–89.

Pfefferbaum A, Rosenbloom M, Rohlfing T, Sullivan EV (2009) Degrada- tion of association

and projection white matter systems in alcoholism detected with quantitative fiber

tracking. *Biological Psychiatry*, *65*, 680–690.

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 and Experimental Research*, *21*, 521–9.

Pokhrel, P., Herzog, T.A., Black, D.S., Zaman, A. Riggs, N.R., & Sussman, S. (2013). Adolescent neurocognitive development, self-regulation, and school-based drug use prevention. *Prev Sci*, *14*, 218-228.

Rogers, B.P., Parks, M.H., Nickel, M.K., Katwal, S.B., & Martin, P.R. (2012). Reduced fronto-cerebellar functional connectivity in chronic alcohol patients. Alcoholism: Clinical and Experimental Research, 36, 294-301.

Scherling, C., Collins, B., MacKenzie, J., Bielajew, C., Smith, A.M. (2012). Pre-chemotherapy differences in response inhibition in breast cancer patients compared to controls: An fMRI study. *Journal of Clinical and Experimental Neuropsychology, 34*, 543-560.

Shenoy, P., and Yu, A. (2012). Strategic impatience in Go/NoGo versus forced-choice decision-making. *Advances in Neural Information Processing Systems*, *25*, 2132–2140.

Smith, A.M., Walker, L.A.S., Freedman, M.S., DeMeulemeester, C., Hogan, M.J., Cameron, I. (2009). fMRI investigation of disinhibition in cognitively impaired patients with multiple sclerosis. *Journal of the Neurological Sciences*, *281*, 58-63.

Smith, A.M., Zunini, R.A.L., Anderson, C.D., Longo, C.A., Cameron, I., Hogan, M.J. & Fried, P.A. (2011). Impact of marijuana on response inhibition: an fMRI study in young adults. *Journal of Behavioural and Brain Sciences*, *1*, 24-33.

Squeglia, L. M., Pulido, C., Wetherill, R. R., Jacobus, J., Brown, G. G., & Tapert, S. F. (2012). Brain response to working memory over three years of adolescence: influence of initiating heavy drinking. *Journal of Studies on Alcohol and Drugs*, *73*(5), 749–60.

Stevens, M.C., Kiehl, K.A., Pearlson, G.D., & Calhoun, V.D. (2007). Functional networks underlying inhibition in adolescents and adults. *Behavioural Brain Research, 181,* 12-22.

Stevens, M.C., Kiehl, K.A., Pearlson, G.D., & Calhoun, V.D. (2009). Brain network dynamics

during error commission. *Human Brain Mapping*, *30*, 24–37.

Sullivan, E.V., Harris, R.A., & Pfefferbaum, A. (2010). Alcohol's effects on brain and

behaviour. *Alcohol Research & Health*, *33*, 127-143.

Tamm, L., Menon, V., & Reiss, A.L. (2002). Maturation of brain function associated with

response inhibition. *American Academy of Child and Adolescent Psychiatry, 41,* 1231-1238.

Verbaten, M.N. (2009). Chronic effects of low to moderate alcohol consumption on structural

and functional properties of the brain: beneficial or not? *Human Psychopharmacology,

24, 199-205.*

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

Psychological Corporation).

Welch, K.A., Carson, A., & Lawrie, S.M. (2013). Brain structure in adolescents and young

adults with alcohol problems: systematic review of imaging studies. Alcohol and

Alcoholism, 48, 433-444.

Wetherill, R. R., Squeglia, L. M., Yang, T. T., & Tapert, S. F. (2013). A longitudinal

examination of adolescent response inhibition: neural differences before and after the

initiation of heavy drinking. *Psychopharmacology*, *230*(4), 663–71. doi:10.1007/s00213-

013-3198-2.

World Health Organization (WHO). (2007). *WHO Expert Committee on Problems Related to

Alcohol Consumption – 2nd Report*. Geneva, Switzerland: WHO Press.

Wrege, J., Schmidt, A., Walter, A., Smieskova, R., Bendfeldt, K., Radue, E.W., Lang, U.E., &

Borgwardt, S. (2014). Effects of Cannabis on Impulsivity: A systematic review of

neuroimaging findings. *Current Pharmaceutical Design, 20,* 2126-2137.

Wright, L., Lipszyc, J., Dupuis, A., Thayapararajah, S.W., & Schachar, R. (2014). Response

inhibition and psychopathology: A meta-analysis of Go/No-Go task performance. Journal

of Abnormal Psychology, 123, 429-439.

Yamaguchi, S., Zheng, D., Oka, T., Bokura, H. (2008). The key locus of common response

inhibition network for No-Go and Stop Signals. Journal of Cognitive Neuroscience,

20(8), 1434-1442.

Zhao, X., Liu, X., Zan, X., Jin, G., & Maes, J.H.R. (2016). Male smokers and non-smokers

response inhibition in Go/No-Go tasks: Effect of three task parameters. *PLOS One,*

DOI:10.1371/journal.pone.0160595.

Table 1. Environmental and IQ variables for alcohol users and controls.

Variable	Users $n = 17$ Mean (SE)	Controls $n = 11$ Mean (SE)	Results
Family income	31769 (14606)	35179 (18875)	$F(1,28) = 0.25$, $p< 0.62$
Average parental education[*]	2.62 (0.92)	2.72 (0.93)	$F(1,28) = 0.09$, $p< 0.77$
Number of years of schooling	11.23 (1.17)	11.36 (0.50)	$F(1,28) = 0.12$, $p< 0.73$
WAIS full scale IQ	111.00 (13.55)	117.46 (11.92)	$F(1,28) = 1.51$, $p< 0.23$
Connors (conduct problems)	0.34 (0.79)	0.31 (0.83)	$F(1,28) = 0.01$, $p< 0.93$
Connors (learning problems)	0.42 (0.95)	0.28 (1.26)	$F(1,28) = 0.10$, $p< 0.76$
Connors (impulsivity-hyperactivity)	-0.07 (1.22)	0.14 (0.75)	$F(1,28) = 0.25$, $p< 0.62$
Connors (anxiety)	0.18 (1.14)	0.45 (1.28)	$F(1,28) = 0.31$, $p< 0.59$
Connors (psychosomatic problems)	0.59 (1.37)	0.45 (1.50)	$F(1,28) = 0.05$, $p< 0.82$

No significant differences were observed between the groups for any variable.

[*]Education was coded as 1 – did not finish high school, 2- graduated from high school, 3 – graduated from college or university, 4- obtained a post graduate degree

Table 2. Drug exposure based on current alcohol use grouping.

Drug exposure	Users $n = 17$[a] Mean (SE)	Controls $n = 11$ Mean (SE)	Results
Current marijuana (joints/week)	5.16 (10.63)	2.00 (4.26)	$F(1,28) = 0.95$, $p< 0.34$
Current nicotine (cigarettes/day)	3.38 (5.87)	2.13 (3.89)	$F(1,28) = 0.42$, $p< 0.52$
Prenatal alcohol (AA/day)[b]	0.26 (0.29)	0.13 (0.21)	$F(1,28) = 1.75$, $p< 0.20$
Prenatal caffeine (mg/day)	86.71 (100.22)	97.17 (117.09)	$F(1,28) = 0.67$, $p< 0.80$
Prenatal marijuana (joints/week)	2.71 (4.32)	6.78 (15.40)	$F(1,28) = 1.08$, $p< 0.31$
Prenatal nicotine (cigarettes/day)	6.05 (9.22)	7.13 (11.74)	$F(1,28) = 0.08$, $p< 0.78$

[a] Current alcohol user (mean 4.72 [SE 0.73] drinks/week)

[b] Ounces of absolute alcohol per day.

Table 3. Performance data for the two conditions based on alcohol use.

| Performance measure	Users $n = 17$ Mean (SE)	Controls $n = 11$ Mean (SE)	Results
Errors of commission 'Press for X'	0.81 (0.22)	0.63 (0.20)	$F(4, 24) = 0.71$, $p=0.59$
Errors of commission 'Press for all letters except X'	4.56 (0.99)	3.36 (1.07)	$F(4, 24) = 0.49$, $p= 0.74$
Errors of omission 'Press for X'	0.13 (0.09)	0.00 (0.00)	$F(4, 24) = 1.40$, $p= 0.269$
Errors of omission 'Press for all letters except X'	0.19 (0.10)	0.18 (0.12)	$F(4, 24) = 0.98$, $p= 0.44$
Reaction time 'Press for X'	0.38 ms (0.01)	0.41 ms (0.02)	$F(4, 24) = 1.91$, $p=0.15$
Reaction time 'Press for all letters except X'	0.39 ms (0.01)	0.43 ms (0.02)	$F(4, 24) = 1.89$, $p= 0.14$
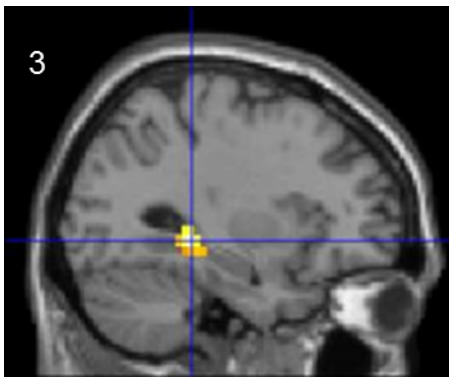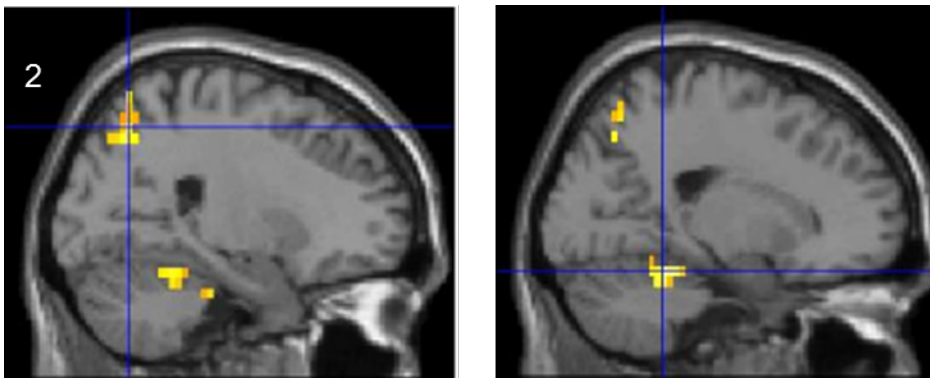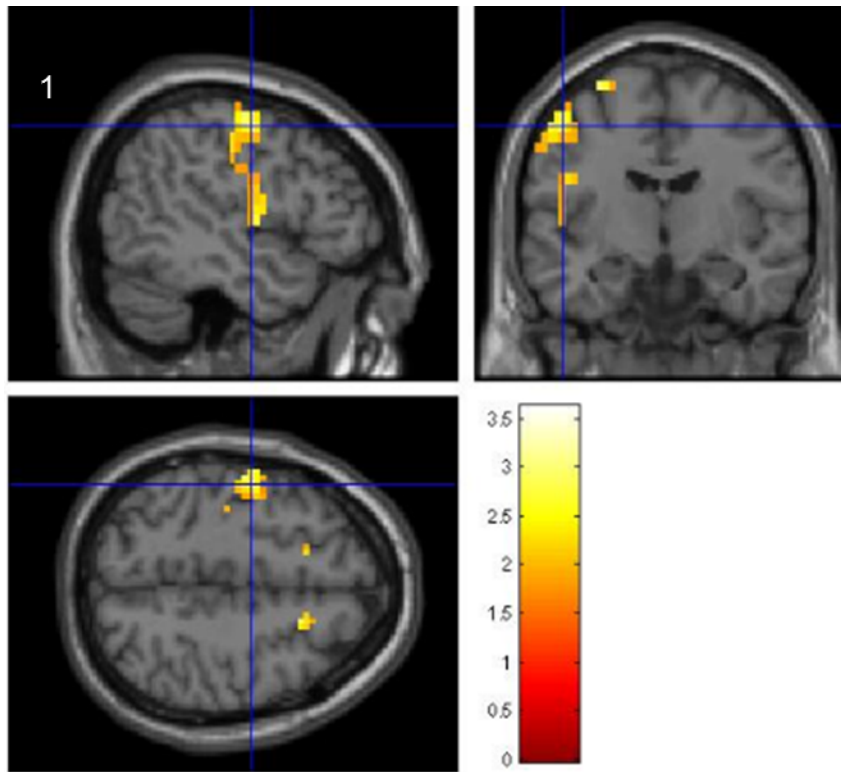
Figure 1. Blue cross hairs highlight areas where there was significantly more activity in current alcohol users compared to controls in the response inhibition 'Press for all letters except X' condition – 'Press for X' condition contrast. 1) **Cluster 1: 1115 voxels, (P<.000)**: Superior Frontal Gyrus (-30, 51, 30), Precentral Gyrus (-39, -18, 65) & (-39, -27, 60); 2) **Cluster 2: 595 voxels, (P<.005)**: Right Superior Parietal Lobe (18, -51, 75): Right Cerebellum (15, -39, -20); 3) **Cluster 3: 248 voxels, (P<.05)**: Left Hippocampus (-30, -36, -5), Parahippocampal Gyrus (-24, -54, 0).

Chapter 4:

General Discussion

Given the high prevalence of adolescent substance use in Canada (Canadian Alcohol and Drug Use Monitoring Survey, 2012), typically beginning with alcohol, it is important to broaden our understanding of the risks and implications of regular alcohol use on neurophysiological functioning in the developing brain. Of particular interest is that the prefrontal cortex (PFC) continues to mature well into early adulthood, after regular alcohol use has been initiated for many teens (Baarendse, Counotte, O'Donnell, & Vanderschuren, 2013; Casey et al., 2005; Paus et al., 2008). This development includes changes in white matter, such as increased myelination to increase speed of neural communication (Arain et al., 2013; Ladouceur, Peper, Crone, & Dahl, 2012), as well as elimination of weak connections through synaptic pruning to optimize function (Blakemore & Choudhury, 2006; Jacobus & Tapert, 2013). Importantly, even minor disruption to these critical developmental processes can result in long-term functional changes, in terms of both cognition and neurophysiology. The PFC, with its connections to other brain regions, is thought to underlie essential higher order cognitive processes, including executive functions (e.g. decision-making, impulse control, attentional resources) (Pokhrel et al., 2013; Robins and Arnsten, 2009; Siddiqui, Chatterjee, Kumar, Siddiqui, & Goyal, 2008). This may, in turn, also have significant implications for social and emotional functioning (Jacobus & Tapert, 2013).

The majority of current research on alcohol's influence on executive functions and associated neurophysiology has focused on the effects of moderate to heavy alcohol consumption, despite knowledge that alcohol related brain damage occurs along a dosage continuum (Paul et al. 2008). Moreover, much of the literature has had inadequate control of potentially confounding lifestyle variables, such as prenatal drug exposures, intellectual functioning, and socioeconomic status, making it difficult to definitively isolate the effect of alcohol on executive functions. In light of our limited understanding of and the general lack of

research examining the effect of low quantities of alcohol, the purpose of the research presented in this dissertation was to address these important gaps in the literature. Through the use of a highly controlled sample of participants, the present research provides preliminary insight into the unique effects of regular consumption of low amounts of alcohol on executive functions and associated brain processes. Findings were presented in two manuscripts examining cognitive interference and response inhibition, respectively.

The first study examined the impact of regular low-level alcohol use on the neurophysiology of cognitive interference. Brain activity for low-level alcohol users and controls was measured using fMRI during completion of the Counting Stroop task. The major findings of this study were that, although there were no significant performance differences between groups observed on cognitive interference, low-level alcohol users showed significantly more regional brain activation while completing the task. This included increased activation in the cerebellum, thalamus, fusiform gyrus, superior parietal lobule, precuneus, as well as significant differences in the PFC, cingulate gyrus and superior temporal gyrus. Importantly, this includes brain areas that are not typically associated with neural processes underlying cognitive interference. This noteworthy difference in neurophysiology between groups may be reflective of compensatory strategies in the brain, in which the brain is recruiting additional brain regions in order to potentially compensate for underlying neural deficits that may be attributable to alcohol use.

The second study investigated how the neurophysiology of response inhibition in young adults may be affected by low-level alcohol use. Using fMRI, low-level alcohol users and controls completed a Go/No-Go task. Similar to the results of the first study, there were no significant performance differences between groups during the performance of the Go/No-Go task. However, the primary findings found significantly more brain activity in the left

hippocampus, parahippocampal gyrus, superior frontal gyrus, precentral gyrus, right superior

parietal lobule, and the cerebellum in users compared to controls. These results showed that

despite only low amounts of regular alcohol use, there was still a significant impact on the

neurophysiology of response inhibition of young adults. Together, these two studies suggest that

regular low-level alcohol use does have a significant impact on the neurophysiological

functioning of the brain in young adults, which may be reflective of underlying deficits that are

compensated for by recruitment of additional brain regions to perform the tasks.

     Together, both studies showed a pattern of increased neurophysiology in regions of the

brain that are normally required in cognitive interference and inhibitory control. In addition,

there was also significant activity in other areas of the brain that are not typically associated with

these distinct processes. These additional areas may be reflective of compensatory mechanisms

as a result of neural changes related to regular alcohol use during a critical period of

neurocognitive development (Chanraud & Sullivan, 2014; Silveri et al., 2011). For example, in

the Counting Stroop, the largest differences in brain activity occurred in the fusiform gyrus, a

region related to visual object processing and attention, as well as the precuneus, which is

involved in visual information processing (Cavanna, & Trimble, 2006; Tallon-Beaudry et al.,

2005). This may reflect a greater need for attentional resources, reflected by increased neural

activity, among the alcohol users in order to adequately complete this type of processing. In the

Go/No-Go task, additional activity was observed primarily in the hippocampus and

parahippocampal gyrus, which are primarily related to memory related functions (Hicklin et al.,

2011). As there is a working memory component to the task itself (remembering instructions), it

may be the case that there was strain on these resources while performing the task resulting in

the increased neurophysiological response. Importantly, all three regions have been shown to be

sensitive to the effects of alcohol (Beresford et al., 2006; Feldstein-Ewing, Sakhardande, &

Blakemore, 2014).

However, in contrasting the two studies, it may be expected that both studies would show

more consistency among the additional areas activated during performance of the tasks. This

raises the question of whether regular consumption of low-amounts of alcohol has a global

impact on neural functioning, or whether it results in a more subtle, process specific effect. The

results of the current two studies seem to suggest the latter. For example, while there is some

degree of overlap between the two tasks, such as both containing an element of inhibitory

control, both tasks are tapping into two distinct constructs that require different types of

information processing. More specifically, in the Go/No-Go task, this occurs through inhibiting a

prepotent response (i.e. learning to press for 'X'), whereas the Counting Stroop task requires

response interference control in the face of distracting stimuli (i.e. counting the number of

stimuli in the face of distracting/potentially incongruent written number words) (Bush et al.,

2006; Wright et al., 2014).

As a result, it may be the case that distinct executive processes require distinct routes of

neural compensation depending on the nature of the demands. Moreover, because executive

functions normally involve the PFC as well as its connections with secondary support areas, it

may also be the case that certain types of executive processes are more vulnerable or sensitive to

alcohol related damage. Ultimately, this raises more questions than answers that can be provided

by the preliminary nature of the current studies and further research into the effects of low-level

alcohol use and the neural compensation hypothesis is clearly required.

Limitations and future directions

The studies included in this dissertation involved several limitations that are important to acknowledge when considering the present findings. Given the small number of participants used in the alcohol users and controls groups, replication of the results in future research is critical. Moreover, as with many research samples, the lack of diversity in the OPPS sample used in the present studies limits generalizability of these findings. For example, the current participants are primarily Caucasian, middle class socioeconomic status and do not represent a higher risk potential for substance misuse.

As such, they may not be fully representative of the broader Canadian society, particularly as many vulnerability factors could have a significant impact on brain functioning. For example, although children of alcoholics have a higher relative risk of future substance abuse, depending on their affective circuitry, some children may be more resilient than others. This has been shown with altered activity in the orbitofrontal cortex and the insula during emotional monitoring between more vulnerable and more resistant individuals (Heitzeg, Nigg, Yau, Zubieta, & Zucker, 2008).

The strength of the tasks used in the present studies is that both are well-established measures of response inhibition and cognitive interference, respectively. However, the relative simplicity of these constructs has certain limitations to fully determine the definitive cause of the differences between low-level alcohol users and controls. While the observed differences in neurophysiology between groups are likely attributable to compensatory mechanisms (Chanraud & Sullivan, 2014; Silveri et al. 2011; Sullivan, Harris, and Pfefferbaum, 2010), the use of tasks with greater levels of complexity may have helped clarify this, such as the Multisource Interference Task for cognitive interference or the Stop Signal Task for response inhibition

(Bush & Shin, 2006; Li, Huang, Constable, & Sinha, 2006; Roberston et al., 2014). For example, this increased complexity could help determine if there is a certain level of difficulty where the brain is no longer able to compensate and performance declines. In addition, it would have been helpful to rule out potential structural differences that may account for differences in neural processing, though this was not possible in the present study given the low-quality of the structural scans acquired.

Consequently, future studies involving the OPPS sample can build upon the limitations of the present study in a number of ways. For example, reimaging the participants using the same tasks, as well as the addition of a more complex form of inhibitory control and cognitive interference, will help clarify: 1) whether the observed differences between alcohol users and controls is long-lasting and/or worse over time; and 2) whether alcohol users' performance will plateau with increased difficulty. This will provide a more in depth understanding of whether differences in neurophysiology between alcohol users and non-drinkers are truly related to compensatory mechanisms. Imaging the same participants again would also allow for further MRI techniques to be added to the protocol, including diffusion tensor imaging and higher quality anatomical scans. These results would help to understand whether potential differences in functional results are related to structural differences in terms of brain volume or white matter integrity.

Given the limitations of the OPPS sample itself, future longitudinal studies could further elucidate the relationship between executive functions and alcohol use by recruitment of a much larger, more diverse sample of individuals in order to test the neural compensation hypothesis more accurately. For example, recruiting individuals from different socioeconomic statuses, as well as ethnic and racial backgrounds would provide a broader context and also allow

researchers to evaluate whether particular risk factors are linked to potential differences in

neurophysiology. In an ideal study, similar to the OPPS, participants would be followed

longitudinally, but would be imaged on a 3 Tesla scanner, in adolescence, prior to initiation of

substance use, and re-imagined in young adulthood using both functional imaging during

executive functioning and structural imaging to examine brain volume and white matter

integrity.

Ideally, the sample would be large enough to compare non-drinkers to low, moderate, and

heavy drinkers. This would allow researchers to establish whether: 1) there are pre-existing

differences in functional brain activity or structural integrity; 2) what effect low-level alcohol use

has relative to moderate and heavy consumption; 3) if neural compensatory strategies are being

used and if so, whether at a certain level of task difficulty or amount of drinking performance is

impacted relative to controls; 4) if the effect of alcohol use at different levels of drinking is

pervasive across individuals or whether it is amplified by other vulnerability factors. Conducting

a follow-up study later in adulthood would also be beneficial as drinking at different severity

levels during adolescence and young adulthood may have a long-lasting impact on the brain and

neural processing. More specifically, it could help clarify whether these differences are

maintained or worsen with continued drinking, as well as whether there is a "return to baseline"

or improved functioning among alcohol users whose drinking becomes irregular or discontinued

with age.

In addition, it would be helpful to have a sample large enough to study whether there are

gender differences that contribute to the changes observed in response to regular alcohol use,

given the differences in blood concentration and metabolism of alcohol between males and

females. Moreover, it would be helpful to quantify the pattern of alcohol use (e.g. drinking one

drink every day versus 7 drinks one day per week), as well as the type of alcohol (e.g. beer, wine, spirits) as different types of alcoholic beverages have different concentrations of alcohol content (e.g. 5% versus 40%), which may have important implications for its effect on the brain. Ultimately, this would allow us to understand the true vulnerability of the developing brain and the potential damage of alcohol related use along a dosage continuum.

Implications of present findings

The findings of the present studies provide preliminary insight for future public awareness and interventions aimed at youth in order to foster a greater understanding of the risks and potential consequences of alcohol use during neurocognitive development once further research has been conducted. Awareness of the influence that alcohol has on the brain, even in low quantities, is important in allowing individuals to make more informed decisions regarding their substance use and the potential perils associated with it. In particular, this may be most beneficial for youth at higher risk for initiation of alcohol use and progression to dependence, such as individuals with a family history of alcohol dependence, conduct disorder, attention deficit hyperactivity disorder, anxiety issues, parental divorce, nicotine dependence, and marijuana use (Sartor et al., 2007). Preventative initiatives through education should be implemented early and on an ongoing basis.

Conclusions

The purpose of the present dissertation was to provide a preliminary understanding of the potential risks associated with regular low-level alcohol use on the neurophysiology of important aspects of executive functions in young adults. Using fMRI in a sample of young adults recruited from the longitudinal OPPS sample permitted examination of the unique contribution of low

amounts of alcohol on brain functioning by controlling for a number of potentially confounding

lifestyle variables, unlike previous research in this area. Taken together, the findings of both

manuscripts found that regular alcohol use, even in small quantities, has a significant impact on

neurophysiological functioning during cognitive interference and response inhibition.

References

Ahmadi, A., Pearlson, G.D., Meda, S.A., Dager, A., Potenze, M.N., Rosen, R., Austad, C.S., Raskin, S.A., Fallahi, C.R., Tennen, H., Wood, R.M., & Stevens, M.C. (2013). Influence of alcohol use on neural response to Go/No-Go task in college drinkers. *Neuropsychopharmacology*, 38, 2197-2208.

Amaro, E., & Barker, G.J. (2006). Study design in fMRI: Basic principles. *Brain and Cognition, 60,* 220-232.

Ames, S.L., Wong, S.W., Bechara, A., Cappelli, C., Dust, M., Grenard, J.L., & Stacy, A.W. (2014). Neural correlates of a Go/No-Go task with alcohol stimuli in light and heavy drinkers. *Behavioural Brain Research*, 274, 382-389.

Anderson, B. M., Stevens, M. C., Meda, S., Jordan, K., Calhoun, V. D., & Pearlson, G. D. (2011). Functional Imaging of Cognitive Control During Acute Alcohol Intoxication. *Alcoholism, Clinical and Experimental Research*, *35*(1), 156–165.

Arain, M., Haque, M., Johal, L., Mathur, P., Nel, W., Rais, A., Sandhu, R., & Sharma, S. (2013). Maturation of the adolescent brain. *Neuropsychiatric Disease and Treatment*, *9*, 449-461.

Arntzen, K. A., Schirmer, H., Wilsgaard, T. & Mathiesen, E. B. (2010) Moderate wine consumption is associated with better cognitive test results: a 7 year follow up of 5033 subjects in the Tromso Study. *Acta Neurol. Scand. Suppl, 190,* 23-29.

Baarendse, P.J., Counotte, D.S., O'Donnell, P., & Vanderschuren, L.J. (2013). Early social experience is critical for the development of cognitive control and dopamine modulation of prefrontal cortex function. *Neuropsychopharmacology, 38,* 1485-1494.

Banich, M. T., Crowley, T. J., Thompson, L. L., Jacobson, B. L., Liu, X., Raymond, K. M., &
    Claus, E. D. (2007). Brain activation during the Stroop task in adolescents with severe
    substance and conduct problems: A pilot study. *Drug and Alcohol Dependence*, *90*, 175-
    182.

Barinaga, M. (1997). Neuroscience: What makes brain neurons run? *Science, 276*, 196-198.

Barnett, R., Maruff, P., Vance, A., Luk, E.S.L., Costin, J., Wood, C., & Pantelis, C. (2001).
    Abornormal executive function in attention deficit hyperactivity disorder: the effect of
    stimulant medication and age on spatial working memory. *Psychological Medicine, 31,*
    1107-1115.

Beresford, T.P., Arciniegas, D.B., Alfers, J., Clapp, L., Martin, B., Du, Y., Liu, D., Shen, D., &
    Davatzikos, C. (2006). Hippocampus volume loss due to chronic heavy drinking.
    Alcoholism: clinical and experimental research, *30*, 1866-1870.

Biederman, J., Monuteaux, M.C., Doyle, A.E., Seidman, L.J., Wilens, T.E., Ferrero, F., Morgan,
    C.L., & Faraone, S.V. (2004). Impact of executive function deficits and attention-
    deficit/hyperactivity disorder (ADHD) on academic outcomes in children. *Journal of
    Consulting and Clinical Psychology, 72*, 757-766.

Blair, C. (2016). Developmental science and executive function. *Current Directions in
    Psychological Science, 25,* 3-7.

Blakemore, S.J., & Choudhury, S. (2006). Development of the adolescent brain: Implications for
    executive function and social cognition. *Journal of Child Psuchology and Psychiatry, 47,*
    296-312.

Brady, J. (2006). The association between alcohol misuse and suicidal behaviour. *Alcohol &*

      Alcoholism, *41*, 473-478.

Bush, G., Whalen, P. J., Rosen, B. R., Jenike, M. A., McInerney, S. C., & Rauch, S. L. (1998).

      The counting Stroop: an interference task specialized for functional neuroimaging--

      validation study with functional MRI. *Human Brain Mapping*, *6*, 270–82.

Bush, G., & Shin, L.M. (2006) The Multi-Source Inference Task: an fMRI task that reliably

      activates the cingulo-frontal-parietal cognitive attention network. *Nature Protocols, 1,*

      308-313.

Bush, G., Whalen, P. J., Shin, L. M., & Rauch, S. L. (2006). The counting Stroop: a cognitive

      interference task. *Nature Protocols*, *1*, 230–3.

Cabeza, R., & Dennis, N.A. (2013). Frontal lobes and aging: deterioration and compensation.

      Principles of frontal lobe functioning. Oxford University Press, New York.

Canadian Cancer Society's Advisory Committee on Cancer Statistics (2015). *Canadian Cancer*

      Statistics. Toronto, ON: Canadian Cancer Society.

Canadian Centre on Substance Abuse (2012). *Canadian Alcohol and Drug Use Monitoring*

      Survey. Ottawa, ON: Health Canada.

Casey, B.J., Tottenham, N., Liston, C., & Durston, S. (2005). Imaging the developing brain:

      what have we learned about cognitive development? *Trends in Cognitive Science,* 9, 104–

      110.

Cavanna, A.E., & Trimble, M.R. (2006). The precuneus: a review of its functional anatomy and

      behavioural correlates. *Brain, 129,* 564-583.

Chanraud, S., & Sullivan, E.V. (2014). Compensatory recruitment of neural resources in chronic

    alcoholism. *Handbook of Clinical Neurology*, *125*, 369-380.

Chang, L., Yakupov, R., Nakama, H., Stokes, B., & Ernst, T. (2008). Antiretroviral treatment is

    associated with increased attentional load-dependent brain activation in HIV patients.

    Journal of Neuroimmune Pharmacology, 3, 95-104.

Clapp, P., Bheave, S.V., & Hoffman, P.L. (2008). How adaption of the brain to alcohol leads to

    dependence: a pharmacological perspective. *Alcohol Research & Health*, *31*, 310-339.

Cox, W.M., Pothos, E.M., & Hosier, S.G. (2007). Cognitive-motivational predictors of excessive

    drinkers success in changing. *Psychopharmacology*, *192*, 499-510.

Curtin, J.J., Patrick, C.J., Lang, A.R., Cacioppo, J.T., & Birbaumer, B. (2001). Alcohol affects

    emotion through cognition. *Psychological Science, 12,* 527-531.

Crews, F.T., Buckley, T., Dodd, P.R., Ende, G., Foley, N., Harper, C., & Sullivan, E.V. (2005).

    Alcoholic neurobiology: changes in dependence and recovery. *Alcohol Clinical &

    Experimental Research*, *29*, 1504–2513.

Czapla, M., Baeuchl, C., Simon, J.J., Richter, B., Kluge, M., Friederich, H.C., Mann, K.,

    Herpertz, S.C., & Loeber, S. (2017). Do alcohol-dependent patients show different neural

    activation during response inhibition than healthy controls in an alcohol-related fMRI

    go/no-go task? *Psychopharmacology,* DOI 10.1007/s00213-017-4541-9.

De Bellis M.D., Clark, D.B., Beers, S.R., Soloff, P.H., Boring A.M., Hall., J., Kersh, A., &

    Keshavan, M.S. (2000). Hippocampal volume in adolescent-onset alcohol use disorders.

    The American Journal of Psychiatry, *157,* 737-744.

De Bellis, M.D., Narasimhan, A., Thatcher, D.L., Keshavan, M.S., Soloff, P. & Clark, D.B.

(2005). Prefrontal cortex, thalamus, cerebellar volumes in adolescents and young adults

with adolescent-onset alcohol use disorders and comorbid mental disorders. *Alcohol*

Clinical and Experimental Research, 29, 1590-1600.

Deng, W., Aimone, J.B., & Gage, F.H. (2010). New neurons and new memories: how does adult

hippocampal neurogenesis affect learning and memory? *Nature Reviews Neuroscience,*

11, xi339-350.

Diamond, A. (2013). Executive functions. *Annual Review of Psychology, 64,* 135-168.

Donovan, E., Das Mahapatra, P., Green, T.C., Chiauzzi, E., Mchugh, K., & Hemm, A. (2015).

Efficacy of an online intervention to reduce alcohol-related risks among community

college students. *Addiction Research & Therapy, 23,* 437-447.

Doughtery, D.M., Marsh, D.M., Moeller, F.G., Chokshi, R.V., & Rosen, V.C. (2000). Effects of

moderate and high doses of alcohol on attention, impulsivity, discriminability, and

response bias in immediate and delayed memory task performance. *Alcoholism: Clinical*

and Experimental Research, *24*, 1702-1711.

Duncan, R.J., McClelland, M.M., & Acock, A.C. (2017). Relations between executive function,

behavioural regulation, and achievement: Moderation by family income. *Journal of*

Applied Developmental Psychology, 49, 21-30.

Drummond, S.P., Meloy, M.J., Yanagi, M.A., Orff, H.J., & Brown, G.G. (2005). Compensatory

recruitment after sleep deprivation and the relationship with performance. *Psychiatry*

Research, 140, 211-223.

Feldstein-Ewing, S.W., Sakhardande, A., & Blackmore, S.J. (2014). The effect of alcohol on the

adolescent brain: A systematic review of MRI and fMRI studies of alcohol-using youth.

Neuroimage: Clinical, 5, 420-437.

Fernie, G., Peeters, M., Gullo, M. J., Christiansen, P., Cole, J. C., Sumnall, H., & Field, M.

(2013). Multiple behavioural impulsivity tasks predict prospective alcohol involvement in

adolescents. *Addiction*, *108*, 1916–1923.

Friston K.J., Zarahn, E., Josephs, O., Henson, R.N., Dale, A.M. (1999). Stochastic designs in

event-related fMRI. *Neuroimage, 10*, 607-619.

Fuster, J.M. (2006). The cognit: A network model of cortical representation. *International*

Journal of Psychophysiology, 60, 125-132.

Fuster, J.M. (2015). *The prefrontal cortex*. London, UK: Academic Press.

Ganesalingam, K., Yeates, K.O., Taylor, H.G., Chertkoff Walz, N., Stancin, T., & Wade, S.

(2011). Executive functions and social compentence in young children 6 months

following traumatic brain injury. *Neuropsychology, 25*, 466-476.

Giancola, P.R., Josephs, R.A., Parrott, D.J., & Duke, A.A. (2010). Alcohol myopia revisited:

Clarifying aggression and other acts of disinhibition through a disotroed lens.

Perspectives on Psychological Science, 5, 265-278.

Gilpin, N.W., Herman, M.A., & Roberto, M. (2015). The central amygdala as an integrative hub

for anxiety and alcohol use disorders. *Biological Psychiatry, 77,* 859-869.

Grune, B., Piontek, D., Pogarell, O., Grubl, A., Grob, C., Reis, O., Zimmerman, U.S., & Kraus, L. (2017). Acute alcohol intoxication among adolescents – the role of the context of drinking. *European Journal of Pediatrics, 176,* 31-39.

Gyurak, A., Goodkind, M. S., Madan, A., Kramer, J. H., Miller, B. L., & Levenson, R. W. (2009). Do tests of executive functioning predict ability to down regulate emotions spontaneously and when instructed to suppress? *Cognitive, Affective & Behavioral Neuroscience*, *9*, 144-152.

Health Canada (2012). *Canadian Alcohol and Drug Use Monitoring Survey*. Ottawa, ON: Health Canada.

Heikkinen, N., Niskanen, E., Kononen, M., Tolmunen, T., Kekkonen, V., Kivimaki, P., Tanila, H, Laukkanen, E., & Vanninen R. (2016). Alcohol consumption during adolescence is associated with reduced grey matter volumes. *Addiction*, doi: dx.doi.org/10.1111/add.13697.

Heitzeg, M. M., Nigg, J. T., Yau, W.-Y. W., Zubieta, J.-K., & Zucker, R. A. (2008). Affective circuitry and risk for alcoholism in late adolescence: Differences in frontostriatal responses between vulnerable and resilient children of alcoholic parents. *Alcoholism, Clinical and Experimental Research*, *32*, 414–426.

Hicklin, T.R., Wu, P.H., Radcliffe, R.A., Freund, R.K., Goebel-Goody, S.M., Correa, P.R., Proctor, W.R., Lombroso, P.J., & Browning, M.D. (2011). Alcohol inhibition of the NMDA receptor function, long-term potentiation, and fear learning requires striatalenriched protein tyrosine phosphatase. *Proceedings of the National Academy of Science*, 108, 6650 – 6655.

Hines, L.M., & Rimm, E.B. (2001). Moderate alcohol consumption and coronary heart disease: a

    review. *Postgrad Med J*, *77*, 747-752.

Huettel, S. A., Song, A. W., & McCarthy, G. (2009). *Functional magnetic resonance imaging*.

    Sunderland, Mass: Sinauer Associates.

Jacobus, J, & Tapert, S.F. (2013). Neurotoxic effects of alcohol in adolescence. Annual *Review*

    of Clinical Psychology, *9*, doi:10.1146/annurev-clinpsy-050212-185610.

Jarosz, A.F., Colflesh, G.J.H., & Wiley, J. (2012). Uncorking the muse: Alcohol intoxication

    facilitates creative problem solving. *Consciousness and Cognition*, *21*, 487-493.

Johnson, S.B., Blum, R.W., & Giedd, J.N. (2010). Adolescent maturity and the brain: the

    promise and pitfalls of neuroscience research in adolescent health policy. *Jounrnal of*

    Adolescent Health, 45, 216-221.

Karoly, P. (1993). Mechanisms of self-regulation: A systems view. *Annual Review of*

    Psychology, 44, 23-52.

Ladouceur, C.D., Peper, J.S., Crone, E.A., & Dahl, R.E. (2012). White matter development in

    adolescence: The influence of puberty and implications for affective disorders.

    Developmental Cognitive Neuroscience, 2, 36-54.

Li, C.S.R., Huang, C., Constable, R.T., & Sinha, R. (2006). Imaging response inhibition in a

    stop-singal task: Neural correlates independent of signal monitoring and post-response

    processing. *The Journal of Neuroscience, 26,* 186-192.

Longo, C. A., Fried, P.A., Cameron, I., & Smith, A.M. (2013). The long-term effects of prenatal

    nicotine exposure on response inhibition: An fMRI study of young adults.

    Neurotoxicology and Teratology, *39*, 9-18.

Manto, M., Bower, J. M., Conforto, A. B., Delgado-García, J. M., da Guarda, S. N. F., Gerwig,

    M., ... & Molinari, M. (2012). Consensus paper: roles of the cerebellum in motor

    control—the diversity of ideas on cerebellar involvement in movement. *The*

    Cerebellum, *11*, 457-487.

Marhe, R., Luijten, M., van de Wetering, B. J. M., Smits, M., & Franken, I. H. A. (2013).

    Individual Differences in Anterior Cingulate Activation Associated with Attentional Bias

    Predict Cocaine Use After Treatment. *Neuropsychopharmacology*, *38*(6), 1085–1093.

Marinkovic, K., Rickenbacher, E., Azma, S., & Artsy, E. (2012). Acute Alcohol Intoxication

    Impairs Top-down Regulation of Stroop Incongruity as Revealed by BOLD

    fMRI. *Human Brain Mapping*, *33*(2), 319–333.

Martinussen, R., Hayden, J., Hogg-Johnson, D.C., & Tannock, R. (2005). A meta-analysis of

    working memory impairment in children with attention-deficit/hyperactivity disorder.

    Journal of the American Academy of Child & Adolescent Psychiatry, 44, 377-384.

Matthews, P. M., & Jezzard, P. (2004). Functional magnetic resonance imaging. *Journal of*

    Neurology, Neurosurgery, & Psychiatry, *75*, 6-12.

May, D.E.,& Kratochvil, C.J. (2010). Attention-Deficit Hyperactivity Disorder: Recent advances

    in paediatric pharmacotherapy. *Drugs, 70,* 15-40.

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. *Alcohol Clinical and Experimental Research*, *32*, 386-394.

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, 139, 181-190.

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.

Drugs and Alcohol Dependence, *119*, 216-223.

Patrick, M. E., Blair, C., & Maggs, J. L. (2008). Executive function, approach sensitivity, and

emotional decision making as influences on risk behaviors in young adults. *Journal of

Clinical and Experimental Neuropsychology, 30*, 449-462.

doi:10.1080/13803390701523109

Paul, C. A., Au, R., Fredman, L., Massaro, J. M., Seshadri, S., Decarli, C., & Wolf, P. A. (2008).

Association of alcohol consumption with brain volume in the Framingham study. *Archives

of Neurology*, *65*(10), 1363–7. doi:10.1001/archneur.65.10.1363

Paus, T., Keshavan, M., & Giedd, J.N. (2008). Why do many psychiatric disorders emerge

during adolescence? *Nature Reviews Neuroscience, 9,* 947–957.

Penades, R., Catalan, R., Puig, O., Masana, G., Pujol, N., Navarro, V., Guarch, J., Gasto, C.

(2010). Executive function needs to be targeted to improve social functioning with

Cognitive Remediation Therapy (CRT) in schizophrenia. *Psychiatry Research*, *177*, 41-45.

Pessoa L. On the relationship between emotion and cognition. Nat Rev. 2008; 9:148–158.

Pokhrel, P., Herzog, T.A., Black, D.S., Zaman, A., Riggs, N.R., & Sussman, S. (2013). Adolescent neurocognitive development, self-regulation, and school-based drug use prevention. *Prevention Science*, *14*, 218-228.

Rajmohan, V., & Mohandas, E. (2007). The Limbic system. *Indian Journal of Psychiatry, 49,* 132-139.

Ridley, N.J., Draper, B., & Withall, A. (2013). Alcohol-related dementia: an update of the evidence. *Alzheimer's Research & Therapy, 5,* doi: 10.1186/alzrt157.

Robbins, T.W., & Arnsten, A.F.T. (2009). The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. *Annual Reviews in Neuroscience, 32*, 267–287.

Robertson, J.A., Thomas, A.W., Prato, F.S., Johansson, M., & Nittby, H. (2014). Simultaneous fMRI and EEF during the Multi-Source Interference Task. *PLoS ONE, 9,* doi: 10.1371/journal.pone.0114599.

Rubia, K., Overmeyer, S., Taylor, E., Brammer, M., Williams, S.C., Simmons, A., Andrew, C., & Bullmore, E.T. (2000). Functional frontalisation with age: mapping neurodevelopmental trajectories with fMRI. *Neuroscience Biobehavioral Review*, *24*, 13-19.

Sartor, C.E., Lynskey, M.T., Health, A.C., Jacob, T., & True, W. (2007). The role of childhood

    risk factors in initiation of alcohol use and progression to alcohol dependence. *Addiction,*

    102, 216-225.

Saunders, B., Farag, N., Vincent, A. S., Collins, F. L., Sorocco, K. H., & Lovallo, W. R. (2008).

    Impulsive Errors on a Go-NoGo Reaction Time Task: Disinhibitory Traits in Relation to

    a Family History of Alcoholism. *Alcoholism, Clinical and Experimental Research*, *32*,

    888–894.

Sayed, B.A., & French, M.T. (2016). To your health! Re-examining the health benefits of

    moderate alcohol use. *Social Science & Medicine, 167,* 20-28.

Siddiqui, S.V., Chatterjee, U., Kumar, D., Siddiqui, A., & Goyal, N. (2008). Neuropsychology of

    prefrontal cortex. *Indian Journal of Psychiatry*, *50*, 202-208.

Silveri, M.M., Dager, A.D., Cohen-Gilbter, J.E., & Sneider, J.T. (2016). Neurobiological

    signatures associated with alcohol and drug use in the human adolescent brain.

    Neuroscience and Biobehavioral Reviews, *70*, 244-255.

Silveri, M.M., Rogowska, J., McCaffrey, A., Yurgelun-Todd, D.A. (2011). Adolescents at risk

    for alcohol abuse demonstrate altered frontal lobe activation during Stroop performance.

    Alcoholism, Clinical and Experimental Research, *35,* 218-228.

Spear, L.P. (2010). *The behavioral neuroscience of adolescence.* W.W. Norton: New York.

Sullivan, E.V., Harris, A., & Pfefferbaum, A. (2010). Alcohol's effect on brain and behavior.

    Alcohol Research and Health, *33*, 127-143.

Stevens, M.C., Kiehl, K.A., Pearlson, G.D., & Calhoun, V.D. (2007). Functional networks underlying inhibition in adolescents and adults. *Behavioural Brain Research, 181,* 12-22.

Tallon-Beaudry, C., Bertrand, O., Henaff, M.A., Isnard, J., & Fischer, C. (2005). Attention modulates gamma-band oscillations different in the human lateral occipital cortex and fusiform gyrus. *Cerebral Cortex, 15,* 654-662.

Tapert, S. F., Brown, G. G., Baratta, M. V, & Brown, S. A. (2004). fMRI BOLD response to alcohol stimuli in alcohol dependent young women. *Addictive Behaviors*, *29*, 33–50.

Tokuda, K., Izumi, Y., & Zorumski, C.F. (2013). Locally-generated acetaldehyde is involved in ethanol-mediated LTP inhibition in the hippocampus. *Neuroscience Letters*, *537*, 40-43.

Toma, K., & Nakai, T. (2002). Functional MRI in human motor control studies and clinical applications. *Magnetic Resonance in Medical Sciences, 1*, 109-120.

Volkow, N.D., Ma, Y., Zhu, W., Fowler, J.S., Li, J., Rao, M., Mueller, K., Pradhan, K., Wong, C., & Wang, G.J. (2008). Moderate doses of alcohol disrupt the functional organization of the human brain. *Psychiatry Research, 162,* 205-213.

Welsh, M.C., & Pennington, B.F. (1988). Assessing frontal lobe functioning in children: Views from developmental psychology. *Developmental Neuropsychology, 4,* 199-230.

Welsh, M.C., Pennington, B.F., & Grossier, D.B. (1991). A normative-developmental study of executive function: A window on prefrontal function in children. *Developmental Neuropsychology, 7,* 131-149.

Wilcox, C. E., Dekonenko, C. J., Mayer, A. R., Bogenschutz, M. P., & Turner, J. A. (2014).

Cognitive control in alcohol use disorder: deficits and clinical relevance. *Reviews in the Neurosciences*, *25*(1), 1–24.

Wright, L., Lipszyc, J., Dupuis, A., Thayapararajah, S.W., & Schachar, R. (2014). Response

inhibition and psychopathology: A meta-analysis of Go/No-Go task performance. *Journal of Abnormal Psychology*, *123*, 429-439.

Ziegler, D.W., Wang, C.C., Yoast, R.A., Dickinson, B.D., McCaffree, M.A., Robinowitz, C.B.,

& Sterling, M.L. (2005). The neurocognitive effects of alcohol on adolescents and college students. *Preventive Medicine, 40,* 23-32.

Appendix A

Figure 1. Examples of Counting Stroop single trials for the types of stimuli presented in the incongruent and control blocks. During trials for the control condition, common animal names (e.g. cat, dog, bird, or mouse) were presented, whereas in the incongruent blocks, stimuli presented were number names (e.g. one, two, three, or four). In both of the examples below the correct answer would be to indicate a response of 4.
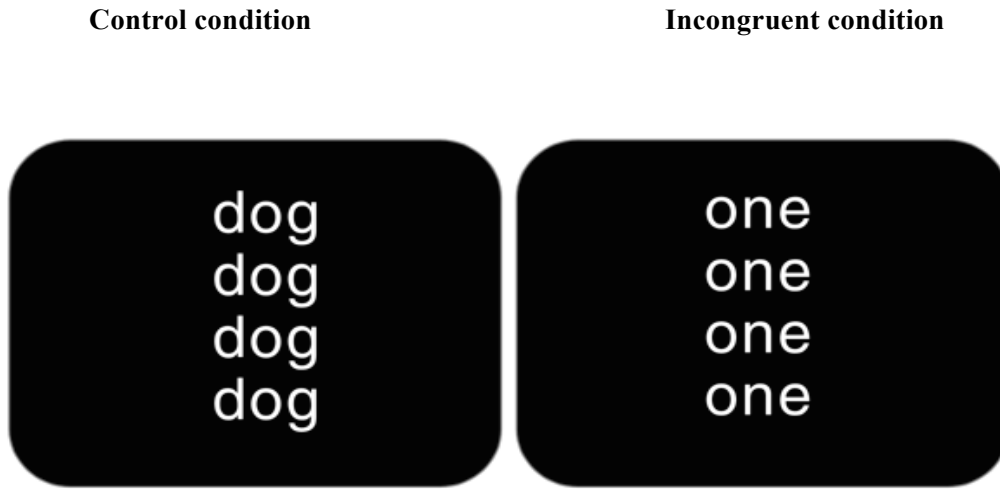
Control condition   **Incongruent condition**

Figure 2. Examples of Go/No-Go single trials for the types of stimuli presented in the experimental (i.e. "Press for all letters except X") and the control condition (i.e. "Press for X").

Control condition   **Experimental condition**